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THE UNIVERSITY OF ALBERTA

DISTRIBUTION AND DYNAMICS OF BARLEY AND FABABEAN ROOTS *IN SITU*

BY

DEO ANAND HEERAMAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

SOIL-PLANT RELATIONSHIPS

DEPARTMENT OF SOIL SCIENCE

EDMONTON, ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled DISTRIBUTION AND DYNAMICS OF BARLEY AND FABABEAN ROOTS *IN SITU* submitted by DEO ANAND HEERAMAN in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in SOIL-PLANT RELATIONSHIPS.

Noorallah G. Juma
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.....
G.J. Taylor

.....
P.H. Crown

Date: July 27, 1992

Dedication

To my parents Iris and Danny, brother Ravi and sister Ranu for their love and continuous support

"You can know more and more about one thing but you can never know everything about one thing: it's hopeless"

Vladimir Nabokov

(Strong Opinions)

Abstract

A study was conducted in the greenhouse to study root distribution and dynamics for barley (*Hordeum vulgare* (L.) cv. Bonanza) and fababean syn. broadbean, fieldbean (*Vicia faba* (L.) cv. Herz Freya) crops. Plants were grown in plywood constructed boxes in a hexagonal arrangement with minirhizotrons installed at a 45° angle. Roots intersecting the wall of the minirhizotron were observed and video-recorded sequentially at various depths (0-70 cm) at specified growth stages. Destructive sampling (core, monolith) was performed at the ripening growth stages in both crops. A significant linear relationship in root length densities (RLD, cm cm^{-3}) between the minirhizotron and core methods was observed in barley (r=0.77) but not in fababean (r=0.52). Measurements of RLD with the monolith method were generally lower for barley than fababean for the upper 40 cm layers of the profile.

Minirhizotron images from different growth stages in the two crops at a particular depth were digitized and registered to each other. Changes in root growth and decomposition were detected by assigning a separate primary color (red, green, blue) to individual scenes representing different growth stages. The scenes were then overlayed to create red-green and red-green-blue color composites. Root length intensity (RLI, cm cm^{-2}) in barley increased from tillering to maximum at heading followed by a sharp decline at ripening. In fababean, RLI increased from leaf growth stage and peaked at early ripening. A growth stage x depth interaction for barley was significant in RLI but was not for fababean. Barley fine root turnover (0.67) was three times greater than fababean (0.23). Lower estimates of RLD were obtained in the top 10 cm layer for both crops at the ripening growth stage compared to the destructive methods. In fababean, higher estimates of RLI were observed at depths >30 cm. Color composites allowed for a visual interpretation and comparison of root dynamics *in situ* for the different crops.

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Table of Contents

1. Introduction 1

   References 5

2. A Comparison of Minirhizotron, Core and Monolith Methods for Quantifying Distribution of Roots in Barley and Fababean 8

   Introduction 8
   Materials and Methods 9
   Results 13
   Discussion 25
   References 31

3. A Color Composite Technique for Detecting Root Dynamics of Barley from Minirhizotron Images 39

   Introduction 39
   Materials and Methods 41
   Results and Observations 46
   Discussion 57
   References 62

4. *In Situ* Detection of Root Dynamics of Barley and Fababean Using a Color Composite Technique 67

   Introduction 67
   Materials and Methods 68
   Results 72
   Discussion 87
   References 91

5. General Discussion and Conclusions 98

   References 107

6. Appendices 109

   Appendix 6.1. Some chemical properties of the Orthic Black Chernozem soil (Typic Cryoboroll) in the plow layer (0-10 cm depth) 110

   Appendix 6.2. Description of specified growth stages in barley and fababean using Zadoks and EPPO code, respectively 110

   Appendix 6.3. Minirhizotron root intersects in barley at specified growth stages 111

   Appendix 6.4. Minirhizotron root intersects in fababean at specified growth stages 112

Appendix 6.5. Total length (cm) of root obtained by minirhizotron, core and monolith methods at ripening growth stages in barley and fababean 113

Appendix 6.6. Root length density (RLD, cm cm^{-3}) obtained by minirhizotron, core and monolith methods at ripening growth stages in barley and fababean 114

Appendix 6.7. Root length intensity (RLI, cm cm^{-2}) obtained by the minirhizotron technique in barley at specified growth stages 115

Appendix 6.8. Root length intensity (RLI, cm cm^{-2}) obtained by the minirhizotron technique in fababean at specified growth stages 116

Appendix 6.9. Percentage root mass distribution obtained by minirhizotron, core and monolith methods at ripening growth stages in barley and fababean 117

Appendix 6.10. Percentage root length distribution obtained by minirhizotron, core and monolith methods at ripening growth stages in barley and fababean 118

Appendix 6.11. Specific root length (cm mg^{-1}) obtained by the monolith method in barley and fababean at ripening growth stages 119

Appendix 6.12. Fractionation of above ground components in barley and fababean at ripening growth stages 120

Appendix 6.13. Root dry weight (mg) in barley and fababean at ripening growth stage using core and monolith methods 121

Appendix 6.14. ANOVA of root length density (cm cm^{-3}) in barley and fababean at ripening growth stages obtained by minirhizotron, core and monolith methods 122

Appendix 6.15. ANOVA of root mass density (mg cm^{-3}) in barley and fababean at ripening growth stages obtained by minirhizotron, core and monolith methods 122

Appendix 6.16. ANOVA of root length intensity (cm cm^{-2}) in barley and fababean obtained by the minirhizotron over the growing season 123

Appendix 6.17. Diameter (mm) and root length (cm) measurements for individual barley and fababean roots 124

List of Tables

II.1. Distribution of root lengths for barley and fababean using Minirhizotron, Core and Monolith methods 14

II.2. Root mass distribution for barley and fababean using Core and Monolith methods. 15

II.3. ANOVA of Root Mass and Root Length Density in barley and fababean. 17

II.4. Equations describing root mass (Y = RMD, mg cm^{-3}) and root length density obtained by the different methods as a function of depth 23

II.5. Relationships between the minirhizotron, core and monolith methods in estimating root mass (mg cm^{-3}) and root length (cm cm-3) density for barley and fababean. 24

III-1. Illustration of change detection by assigning red, green and blue colors to individual images on three (3) dates 46

III-2. Interpretative key for Plate III-3. 52

III-3. Interpretative key for Plate III-4. 52

IV.1 Minirhizotron observation date and growth description of barley and fababean 69

IV.2 Interpretative key for Plate IV-2. 75

IV.3. Root area (%) and turnover dynamics in barley and fababean roots as observed from classified images (Plate IV-3) 81

IV.4. ANOVA of root length intensity (cm cm^{-2}) in barley and fababean 82

IV.5. Chemical analyses of total carbon, nitrogen and phosphorous in barley and fababean roots 85

IV.6. Mean diameter classes for barley and fababean roots 86

IV.7. Specific root length (SRL, m g^{-1}) for barley and fababean measured by the monolith method 86

List of Figures

II.1. Comparison of root length density of barley and fababean by minirhizotron, core and monolith methods..... 18

II.2. Comparison of root mass density of barley and fababean by core and monolith methods.. 19

II.3. Logarithmic depth distribution of root mass density for barley and fababean using minirhizotron, core and monolith methods. 20

II.4. Logarithmic depth distribution of root length density for barley and fababean using minirhizotron, core and monolith methods. 21

III-1. Temporal dynamics of barley roots within a 2.4 cm^2 viewing area obtained at a depth of 66 cm..... 49

IV.1. Root length intensity in barley and fababean at different growth stages for the entire depth (0-70 cm)..... 83

IV.2. Root length intensity distribution with depth (0-70 cm) at different growth stages for barley and fababean..... 84

List of Plates

III-1. Original unregistered digitized images at a depth of 66 cm within 2.4 cm^2 minirhizotron viewing area 48

III-2. Classified single images at a depth of 66 cm depicting root (red) and soil matrix (blue) after a density slice is applied to individual growth stages. 51

III-3. Color composite at a depth of 66 cm after mixing the red and green primary colors of the image display 54

III-4. Color composite at a depth of 66 cm after mixing the red, green and blue primary colors of the image display. 56

IV.1. Original unregistered digitized images in barley and fababean at a depth of 38 cm in barley and 31 cm for fababean within a viewing area of 2.4 cm^2 minirhizotron viewing area 74

IV.2. Color composites in barley and fababean after mixing the red and green primary colors of the image display taken at a depth of 38 cm in barley and 31 cm for fababean within a viewing area of 2.4 cm^2 77

IV.3. Classified single images in barley and fababean after density slicing and small region conversion taken at a depth of 38 cm in barley and 31cm for fababean within a viewing area of 2.4 cm^2 79

Chapter 1. Introduction

Plant roots serve as important organs for water and nutrient uptake, synthesis of growth regulators and storage of carbohydrates. The importance of these functions seems evident, but are still poorly understood compared to the aerial parts of plants (Milthorpe and Ivins, 1966; Persson, 1990). The study of root systems is difficult because they are shielded from direct observation by the soil matrix (McMichael and Taylor, 1987). A further complication arises from the spatial variability of roots and soil properties which are not uniform at the scale of an individual root. Also, roots are not uniformly distributed in the soil with depth or lateral distance from the crown of the plant. Sampling roots is difficult because the horizontal distribution of roots can exhibit extreme anistrophy (van Noordwijk et al., 1985). These difficulties have hampered detailed investigations of plant root systems.

Destructive techniques were the earliest procedures developed for root studies. Detailed description of these techniques have been documented (Böhm, 1979; Schuurman and Goedewaagen, 1971). Although these methods provide quantification of the root system at a time of sampling, they are destructive and preclude possibilities for repeated observations on the same root system. In addition, a large amount of time and labor is required for collecting and processing the samples (Russell, 1977). An underlying assumption of destructive sampling techniques is that the heterogeneity of root distribution from point to point in the field is adequately sampled on each separate occasion and that it remains constant with time. This assumption introduces errors in studying root dynamics because observations must be made at a new location for each date. This can result in either an over- or under-estimate of root dynamics due to soil spatial variability and non-uniform distribution of roots within the soil (Singh et al., 1984).

One procedure which allows direct measurement of root distribution is the observation of roots growing in soil against a transparent panel. These techniques were developed to replace destructive techniques. Root growth can be observed in rhizotrons but these devices are costly to build and microclimatic conditions can be significantly different from adjacent field plots (Taylor et al., 1990). The minirhizotron technique is a variation of the rhizotron in that roots are observed growing in soil behind a clear material. This technique involves installing clear tubes (acrylic or glass) into the soil and lowering a device (fiber optics or video camera) into the tube so that roots can be observed at the soil-tube interface. This is an *in situ* technique that is rapid, non-destructive, non-invasive, provides a great sampling potential and minimizes the point-to-point variation inherent in the destructive methods. Limitations of the technique arises from the difficulty to maintain firm contact between the soil and the wall of minirhizotron as well as the small area of soil viewed at a particular growth stage and depth. This often results in high coefficients of variability (Brown and Upchurch, 1987).

Bates (1937) originally used a mirror and battery lamp mounted on the end of a stick to observe roots intersecting a tube. The mirror-and-stick method limited the usefulness of this technique because roots at depths below about 1 m could not be seen without the aid of a telescope. Results improved with the application of new technologies to observation roots. Waddington (1971) replaced the mirror-and stick arrangement with a coherent fiber optic scope and right angle viewing attachment which improved the quality of the root image. Sanders and Brown (1979) included a 35-mm camera with a fiber-optic duodenoscope to further improve images of the intersecting roots. Dyer and Brown (1980) replaced the 35-mm camera with a black and white video camera. Upchurch and Ritchie (1984) described a system involving a color video camera with a right angle viewing head. This system currently represents the state of the art of observing roots *in situ*. However, the usefulness of this technique is

dependent on whether root growth observed at the soil-tube interface represents root characteristics in the bulk soil (Brown and Upchurch, 1987).

Visual interpretation of images is often a highly subjective process. For this reason, interest has increased in the development of computer imaging and analysis systems for the plant sciences (Yanuka and Elrick, 1985). Videography can be defined as the scientific evaluation of images stored on a video tape. Stutte (1990) discussed several advantages of video images over traditional methods of analyzing and collecting data: (i) data can be collected and analyzed almost simultaneously; (ii) video cassette tapes are inexpensive and provide a permanent record of results; (iii) video equipment is accessible, relatively inexpensive and flexible; and (iv) video imagery provides unique information that is difficult to obtain through alternate methods.

The full advantage of video techniques was realized when video data were linked to a computer. Video image analysis systems developed in almost all branches of experimental science. They have been used to determine land use patterns, evaluate cropping potential, detect plant disease, monitor environmental stress, optimize ground cover management in orchards and map plant communities (Stutte, 1991). Digitization and computer processing of video data has opened virtually an unlimited scope for analysis and applications. The minirhizotron technique offers a chief advantage in that the photographic record is permanent and well adapted to computer image analysis (Brown and Upchurch, 1987). However, data collection and analysis are labor intensive. Lussenhop et al. (1991) suggested that the coupling of minirhizotron data to image analysis offers the exciting prospect of reducing the labor of data analysis, increasing accuracy and allowing direct measurements of rates and turnover.

The overall objective of this study was to use the minirhizotron technique as a quantitative and qualitative tool for investigating root growth dynamics of barley (*Hordeum vulgare* (L.) cv. Bonanza) and fababean syn. broadbean, fieldbean (*Vicia faba* (L.) cv. Herz Freya). The objectives of the study were: (1) to compare the root

length distribution in barley and fababean under greenhouse conditions using minirhizotron, core and monolith methods; (2) to adapt a color composite technique using digital image analyses for *in situ* detection of changes in root growth and decomposition; and (3) to measure the dynamics of root lengths of barley and fababean over a growing cycle.

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Chapter 2. A Comparison of Minirhizotron, Core and Monolith Methods for Quantifying Distribution of Roots in Barley and Fababean

Introduction

Roots are important for anchoring plants, uptake of water and nutrients, storage of carbohydrates and synthesis of growth regulators. Quantification of root growth and distribution is necessary to understand plant-soil interactions, however, root research has been hampered by a lack of good methods and by the amount of time involved in making measurements (Persson, 1990). A number of advances in root sciences have been made in the past 20 years (Whittington, 1969; Carson, 1974; Torrey and Clarkson, 1975; Russell, 1977; Harley and Russell, 1979; Taylor, 1987; Aspects of Applied Biology 22, 1989; Waisel et al, 1991). Recent developments in analytical methods of quantifying roots include nuclear magnetic reasonance imaging, computer assisted tompography, dual gamma probes and minirhizotron technologies (Roots and Soil Methods Conference, St. Louis, Missouri 1990). There is a great need to compare newer nondestructive methods with the older ones.

Removal of specific soil volumes by monolith (1000-5000 cm^3) and core (<1000 cm^3) methods have been traditional methods for field examination of root systems. Detailed description of these methods have been documented by Böhm (1979) and Schuurman and Goedewaagen (1971). Although both methods yield an estimate of root mass and root length, they are destructive, labour intensive and demand a separation of live roots from organic material present in the soil (Russell, 1977). Additionally, the natural spatial variability of root density in the field introduces sampling problems, particularly if individual plants are widely spaced (van Noordwijk et al., 1985).

The minirhizotron technique involves installing clear tubes (acrylic or glass) into the soil and lowering a device (fiber optics or a video camera) into the tube so that roots can be observed at the soil-tube interface. This is an *in situ* technique that is rapid, non-destructive, provides an opportunity for frequent sampling (Brown and Upchurch, 1987) and minimizes the point-to-point variation inherent in the destructive methods (van Noordwijk, 1987). This technique is useful if the root growth observed at the soil-tube interface represents root growth in the bulk soil (Brown and Upchurch, 1987).

Persson (1990) suggested that there is a need to standardize techniques so that results from different experiments may be compared. The evolution of the minirhizotron from a qualitative to a quantitative tool encourages inter-experimental comparisons (Volkmar, 1991). At present, only a few detailed studies have compared methods for quantifying root distribution. The studies by Böhm et al. (1977) and Köpke (1981) were the first attempts in comparing root study methods for soybean and oat root systems under field conditions. However, comparisons with the recently improved minirhizotron technique (Taylor, 1987) are required for other crops and soils. The overall objective of this study was to compare the minirhizotron, core and monolith methods in measuring root length distribution for barley (*Hordeum vulgare* (L.) cv. Bonanza) and fababean syn. broadbean, fieldbean (*Vicia faba* (L.) cv. Herz Freya) under greenhouse conditions.

Materials and Methods

Experimental design

A randomized block experimental design with a split-plot arrangement was used to test the methods of quantifying roots for two crops (2 crops x 3 replications x 3 methods). Six boxes (80 cm long x 80 cm wide x 75 cm deep) were constructed from plyboard (19 mm thick). Walls of the containers were fastened by screws as

described by Schuurman and Goedewaagen (1971). The plant growth media consisted of soil from the Ap horizon of an Orthic Black Chernozem (Typic Cryoboroll) obtained from the Ellerslie Research Station (53° 25' N, 113° 33' W). This soil is naturally endowed with a thick mollic epipedon which has granulat structure, high nutrient and base status, and neutral pH (Crown and Greenlee, 1978). The soil was sifted with a 1 cm mesh sieve to remove rocks, root fragments and straw that may interfere with observations and root mass estimates. The soil in each box was uniformly compacted to a bulk density of 1.2 Mg m^{-3} to a depth of 70 cm (Böhm, 1979) and seeded to barley and fababean. A hexagonal planting arrangement was used to minimize root anistrophy that is evident in row crop rooting patterns and to achieve an equivalent planting density of 204 and 58 plants m^{-2} respectively for barley and fababean. Urea (50 kg N ha^{-1}) and triple superphosphate (20 kg P_2O_5 ha^{-1}) were broadcasted and incorporated into the top 10 cm of the soil used to grow barley. Prior to sowing fababean, seeds were inoculated with *Rhizobium leguminosarum* manufactured by the Nitragin Company (Milwaukee, Wisconsin, U.S.A). Phosphorous fertilizer was applied to fababean at the same rate as to barley.

Plants were grown at 21 °C in a greenhouse at Edmonton (53°N,113°W) without artificial lighting in June, July and August 1990. The day length was between 15 to 16 hours. The 30-year (1951-1980) average monthly radiation during June, July and August ranges between 18 to 22 MJ m^{-2} (Atmospheric Environmental Service, 1982) Growth stages were identified using keys of Zadoks et al. (1974) and EPPO (1987) for barley and fababean, respectively. Imaging and destructive sampling were conducted at ripening growth stages for both crops. This corresponds to Zadoks' scale 87 in barley and EPPO scale of 92 in fababean.

Sampling methods for quantifying roots

Monolith

A soil monolith was obtained using a modified pinboard method. A pinboard measuring 75 cm x 70 cm was constructed from plywood with 8 cm pins screwed in vertical and horizontal rows 5 cm apart. At the ripening growth stage, the container wall was unscrewed and a 10 cm vertical slice of soil was removed from the edge of the container. The pinboard was gently tapped into the soil with a hammer to the fullest extent of the pins (Böhm, 1979). A soil monolith was obtained by pounding a steel metal sheet vertically down the container with a heavy hammer. The pinboard containing the intact soil monolith was removed, laid down flat, and excess soil cut away from the inner face. The monolith was then sectioned vertically into two equal half sections measuring 37.5 cm wide and 70 cm long.

One half-section of the monolith was sectioned horizontally at 10 cm depth increments into soil blocks (37.5 cm wide and 8 cm front to rear and 10 cm deep). For ease of handling, soil blocks for each depth were divided into smaller subsamples, sealed in labelled plastic bags and stored at -20 °C . Roots were separated from soil cores using a hydropneumatic elutriation system (Smucker et al. 1982). Washed samples were stored at 4 °C in 25% ethanol solution until further analysis. Further cleaning involved separation of organic matter and debris by flotation using tweezers. Root lengths were measured by the line intersection method (Newman, 1966) using an automated video image analyzer (Harris and Campbell, 1989). Oven dried weights of roots were recorded after drying at 65 °C for 3 days. Results were expressed as length and mass per volume of sample. In this study, this method was considered to be a standard method because the results were obtained from a large sampling volume.

Soil cores

Soil cores were obtained at 10 cm intervals to a depth of 70 cm using a heavy soil auger (Schuurman and Goedewaagen, 1971). The auger consists of a hollow

cutting tube 8 cm in diameter and 10 cm long with a serrated cutting edge at its base. A hollow shaft is attached to the top of the cutting tube that encloses a movable rod. A plunger is attached to the bottom of the rod which works to force soil cores out of the auger. Soil cores were washed and samples were processed for root length and mass using techniques described under the monolith method. A root length and root mass density was calculated based on the auger volume (502.65 cm^3). Root length and root mass were compared to an equivalent monolith volume (3000 cm^3) by multiplying by 5.97.

Minirhizotron

The minirhizotrons (tubes measuring 58 mm outside diameter, 2 mm thickness and 120 cm length) with permanently sealed bottoms were specially constructed from glass. Transparent plastic grid strips (1 cm x 1 cm) were mounted along the sides of the tube. The tube was installed at a 45° in the center of the box prior to soil filling. The top 100 mm of tube which projected outward from the face of the box was wrapped with black electrical tape. This was done to prevent light from entering the rooting zone (Furuya and Torrey, 1964). When observations were not taken, the tubes were capped with rubber stoppers to further block light, minimize moisture condensation and insulate against temperature gradients.

Roots intersecting the sides of the tube were videorecorded at the ripening growth stages as described by Upchurch and Ritchie (1983). The video-recording system used in this study consisted of a Circon color video camera, MV 9011, a 12.7 cm color video monitor (a Sony color TV with a 38 mm picture tube) and a Sony Video Cassette Recorder (VCR) model EVO-210 (Circon Corporation, Santa Barbara, CA). Images stored on 8 mm tape were retrieved and analyzed for root intersects (N) at 10 cm depth intervals using the counting techniques of Upchurch and Ritchie (1983). The total length of roots at the glass surface for each depth interval was then calculated by inserting the number of intersects into the Newman (1966) equation: $RL = (\pi \times N \times$

A)/2H, where RL = total root length (cm), N = number of intersects, A = area of the observation surface (cm^2) and H = total length of straight lines (cm). A root length density was calculated for each depth increment by using the equation: RLD = RL/(A x D) where RLD is root length density ($cm\ cm^{-3}$) and D = depth of view assumed to be 2 mm (Taylor et al. 1970). Root length was compared to an equivalent monolith volume by multiplying by 535.71.

Statistical analyses

All statistical analyses were performed using PC SAS (Sas Institute, Inc. 1985). The General Linear Model procedure was used for ANOVA and regression analyses. The Least Significant Difference ($p \leq 0.05$) were performed for means separation of significant main effects and interactions. Estimates of root length density (RLD, $cm\ cm^{-3}$) and root mass density (RMD, $mg\ cm^{-3}$) of fababean obtained by different methods indicated skewness of data (W: $p \leq 0.05$) (Shapiro and Wilk, 1965). Data for fababean were transformed as appropriate to establish homogeneity of variance using a Box-Cox procedure (Box and Cox, 1964). This transformation was of the form:

$$Y^* = (Y^{\lambda} - 1)/\lambda \qquad (\lambda \neq 0)$$

where Y and Y* are the original and transformed values, respectively. Values of λ obtained for RLD and RMD transformation in fababean were -1.25 and 0.25, respectively.

Results

Root length and root mass of barley and fababean

Total root length for the entire 70 cm depth was generally higher in barley (159-309 m) than fababean (110-226 m) (Table II.1). Root mass was also greater for barley (999-1818 µg) than fababean (1317-1554 µg) (Table II.2).

Table II.1. Distribution of root lengths† for barley and fababean using Minirhizotron (MR), Core (C) and Monolith (M) methods.

Depth	Root Length			Length Distribution		
Interval	MR	C	M	MR	C	M
(cm)		(m)			(%)	
Barley						
0-10	16.8 (33)c	73.9 (18)b	53.1 (6)a	7.0	23.9	34.2
10-20	41.4 (25)a	37.5 (17)a	15.2 (52)b	17.5	12.1	9.2
20-30	44.2 (38)ab	55.3 (12)a	30.8 (5)b	18.6	17.9	19.8
30-40	38.6 (51)a	43.8 (17)a	7.4 (36)b	15.5	14.2	4.7
40-50	35.8 (31)a	46.7 (61)a	30.1 (34)a	15.3	15.1	18.5
50-60	35.8 (41)a	27.6 (74)ab	16.0 (63)b	14.7	8.9	9.5
60-70	28.8 (59)a	24.2 (55)ab	6.7 (96)b	11.3	7.8	4.1
Total	241.4 (40)a	309.0 (37)b	159.3 (42)c	100.0	100.0	100.0
Fababean						
0-10	24.5 (103)b	46.6 (14)ab	59.1 (8)a	13.5	44.0	48.3
10-20	62.4 (79)a	19.2 (45)b	20.4 (25)b	25.2	17.0	16.4
20-30	12.6 (44)a	12.8 (56)a	13.8 (27)a	5.8	11.2	11.2
30-40	30.9 (46)a	13.8 (64)a	13.0 (32)a	14.1	12.0	10.4
40-50	33.7 (77)a	8.2 (10)b	11.5 (15)ab	14.8	7.7	9.3
50-60	37.2 (57)a	7.2 (35)b	3.6 (16)b	16.6	6.5	2.9
60-70	24.5 (77)a	1.7 (33)b	1.7 (44)b	10.0	1.7	1.4
Total	225.8 (69)a	109.5 (37)b	123.1 (24)ab	100.0	100.0	100.0

†Means with CV in brackets (n=3); means in same row with different letters differ significantly ($P \leq 0.05$).

Table II.2. Root mass† distribution for barley and fababean using Core (C) and Monolith (M) methods.

Depth Interval (cm)	Root Mass C (µg)	Root Mass M (µg)	Mass Distribution C (%)	Mass Distribution M (%)
Barley				
0-10	475 (13)a	367 (13)b	26.1	37.0
10-20	178 (36)a	74 (21)b	9.8	7.4
20-30	333 (12)a	201 (19)b	18.3	20.7
30-40	276 (27)a	41 (27)b	15.2	4.0
40-50	267 (63)a	174 (35)a	14.7	17.3
50-60	143 (60)a	94 (62)a	7.9	9.1
60-70	146 (60)a	48 (99)a	8.0	4.5
Total	1818 (48)a	999 (40)b	100.0	100.0
Fababean				
0-10	531 (28)b	748 (10)a	40.8	48.1
10-20	202 (22)a	202 (19)a	15.3	13.0
20-30	173 (36)a	236 (8)a	13.0	15.2
30-40	170 (54)a	176 (4)a	12.7	11.4
40-50	113 (38)a	127 (16)a	8.6	8.2
50-60	105 (46)a	45 (13)a	7.9	2.9
60-70	22 (44)a	20 (37)a	1.7	1.3
Total	1317 (38)a	1554 (15)a	100.0	100.0

†Means with CV in brackets (n=3); means in same row with different letters differ significantly ($P \leq 0.05$).

Root mass was concentrated in the top 10 cm and decreased with depth of soil. This was more pronounced in fababean than barley. The relative distribution of fababean root mass in the top 10 cm layer were 40 and 48% compared to barley of 26 and 37% estimated by the core and monolith methods, respectively.

The overall mean coefficient of variation (CV) for root length by monolith was lower than the core method in fababean but a reverse trend was observed for barley (Table II.1). Overall mean coefficient of variation (CV) for root mass by monolith was lower than the core method in both crops (Table II.2). For root length, the CV by the core and monolith methods were 37 and 42% in barley compared to 24 and 37% in fababean, respectively. The overall CV by the minirhizotron was higher than that of the monolith for both crops. The overall variation in root length with the minirhizotron in fababean was higher at all depths than in barley. Overall mean CV was 69% in fababean and 40% in barley (Table II.1).

Root length and root mass density of barley and fababean

The effect of method on estimates of RLD and RMD were non-significant for both crops (Table II.3). A method and depth interaction showed that there were significant differences in root distribution of the two crops (Table II.3). Barley RLD estimated by the core and monolith methods decreased with depth. In contrast, estimates by the minirhizotron method peaked in the 20-30 cm depth. In fababean, similar trends were observed except that estimates of RLD by the minirhizotron peaked in the 10-20 cm depth.

There were no significant differences between RLD of fababean measured by core and monolith methods, but they were different for the uppermost 40 cm depth in barley. The RLD estimates using core and monolith methods were highest at 10 cm and ranged between 1.77-2.46 and 1.55-1.97 for barley and fababean, respectively (Fig. II.1). Lower values (0.56 and 0.81 for fababean and barley, respectively) were obtained for the minirhizotron in the top 10 cm layer. However, estimates of RLD in

barley by minirhizotron and core methods were not significantly different below 10 cm soil depth ($p \leq 0.05$) (Fig. II.1). In fababean, the RLD below 30 cm estimated by the minirhizotron method was greater than that estimated by the monolith method. The RLD below 30 cm estimated by the minirhizotron method was similar for each depth. Estimates of RLD in the 40-70 cm depth interval was within the range of 0.82 - 1.03 cm cm^{-3} compared to considerably lower estimates of 0.06 - 0.38 cm cm^{-3} using core and monolith methods.

Table II.3. ANOVA† of Root Length and Root Mass Density in barley and fababean.

Source of		Root Length Density			Root Mass Density	
Variation	DF	Barley	Fababean‡	DF	Barley	Fababean‡
Block (B)	2	ns	ns	2	ns	ns
Method (M)§	2	ns	ns	1	ns	ns
Error A	4			2		
Depth (D)	6	***	***	6	***	***
M x D	12	***	**	6	ns	*
Error B	36			24		

†The difference between means is significant at: *, $p \leq 0.05$; **, $p \leq 0.01$, ***, $p \leq 0.001$; ns, not significant.

‡Statistical analysis was performed on the Box-Cox transformed data.

§Indicates core and monolith for root mass; minirhizotron, core and monolith methods for root length density.

The trends of RMD were similar to those of RLD for core and monolith methods (Fig. II.2). A method and depth interaction was nonsignificant for barley ($p=0.206$) but significant for fababean ($p=0.027$) (Table II.3). In fababean, estimates of RMD by the monolith method decreased more sharply than the core method. Root mass density was concentrated in the top 10 cm layer. Values of root mass density in

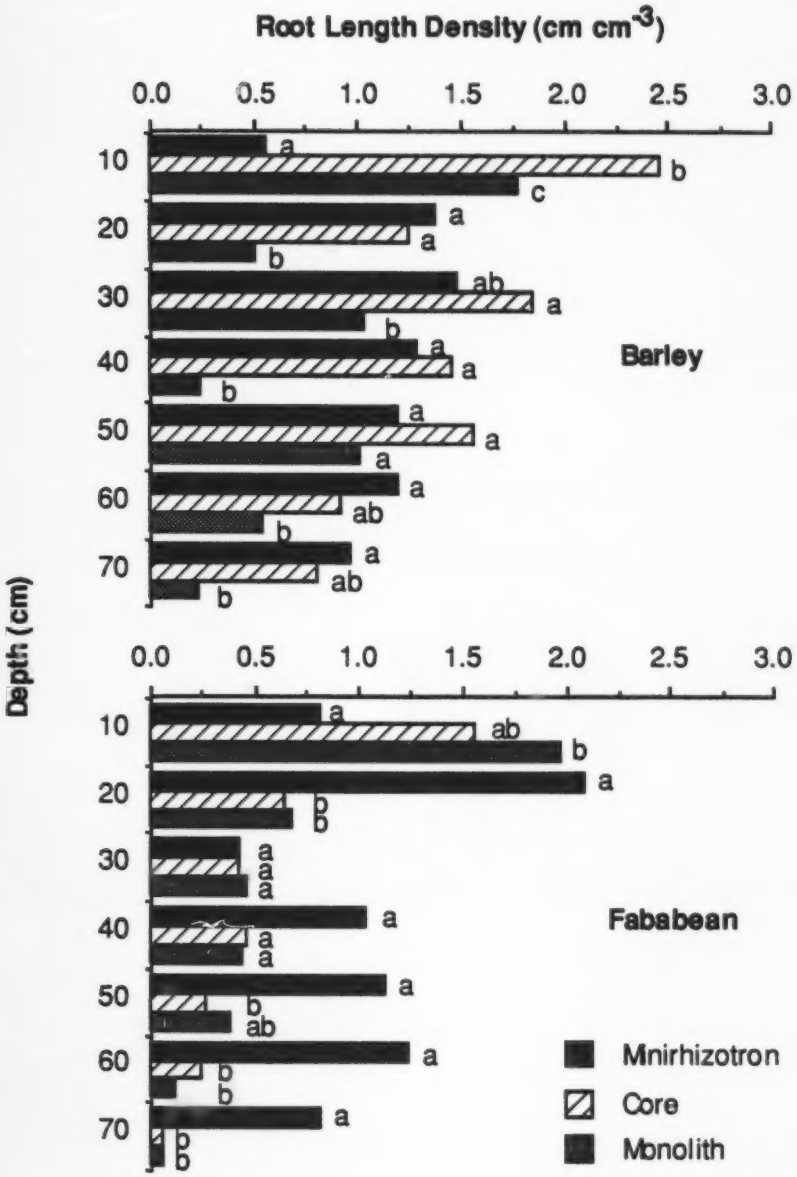

Figure II.1. Comparison of root length density of barley and fababean by minirhizotron, core and monolith methods. The means for each depth bearing different letters are significantly different from one another ($p \leq 0.05$).

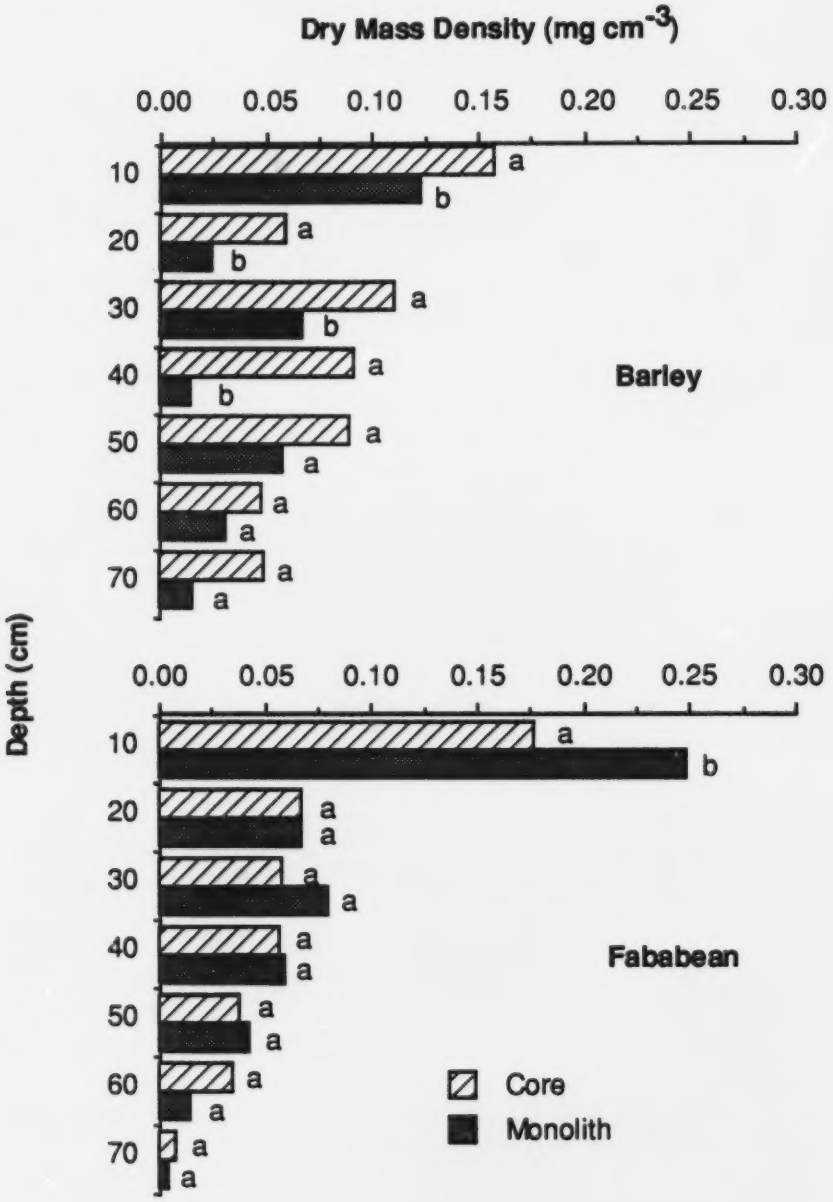

Figure II.2. Comparison of root mass density of barley and fababean by core and monolith methods. The means for each depth bearing different letters are significantly different from each other ($p \leq 0.05$).

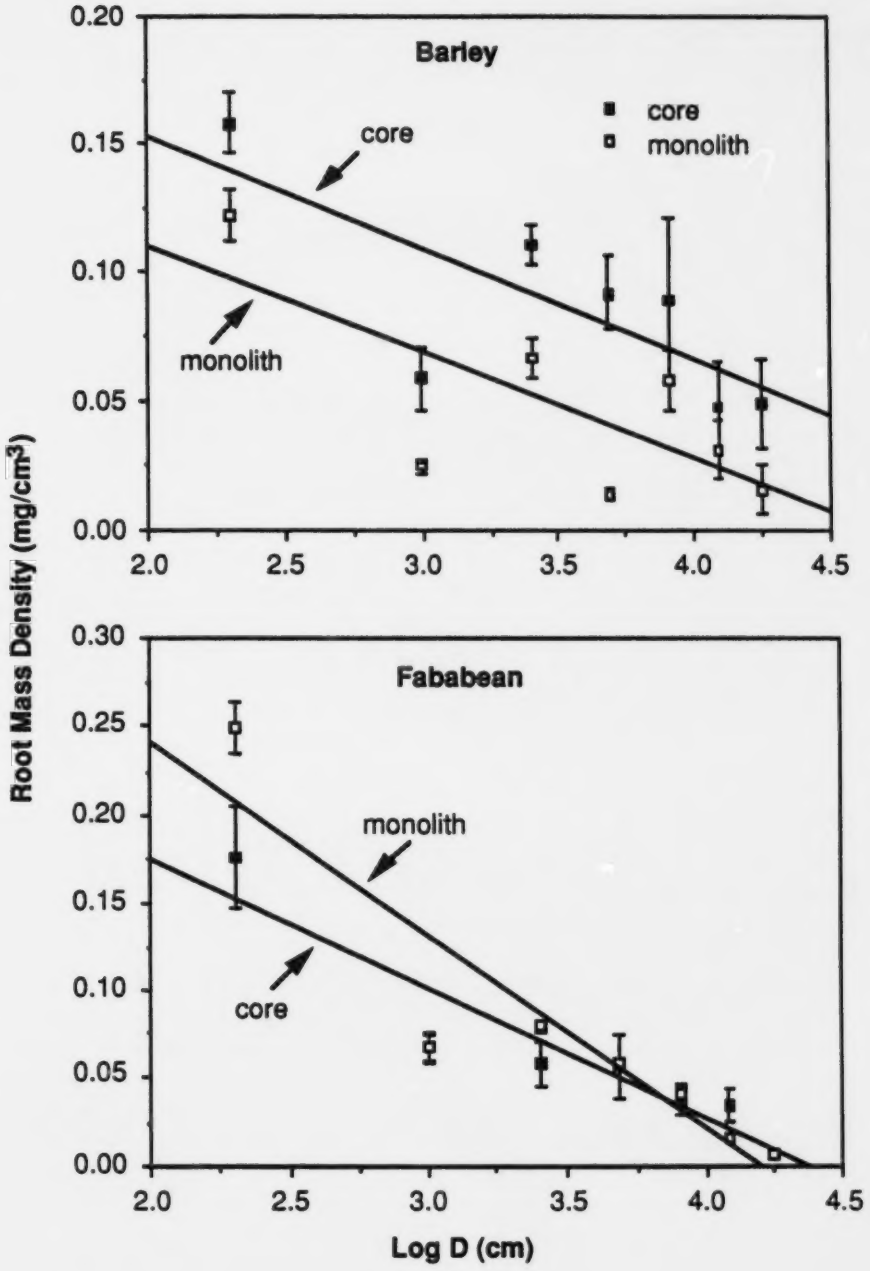

Figure II.3. Logarithmic depth distribution of root mass density (RMD, mg cm^{-3}) for barley and fababean using minirhizotron, core and monolith methods. Vertical bars represent standard error of the mean. Each point is a mean of 3 replicates. Regression equations are given in Table II.4.

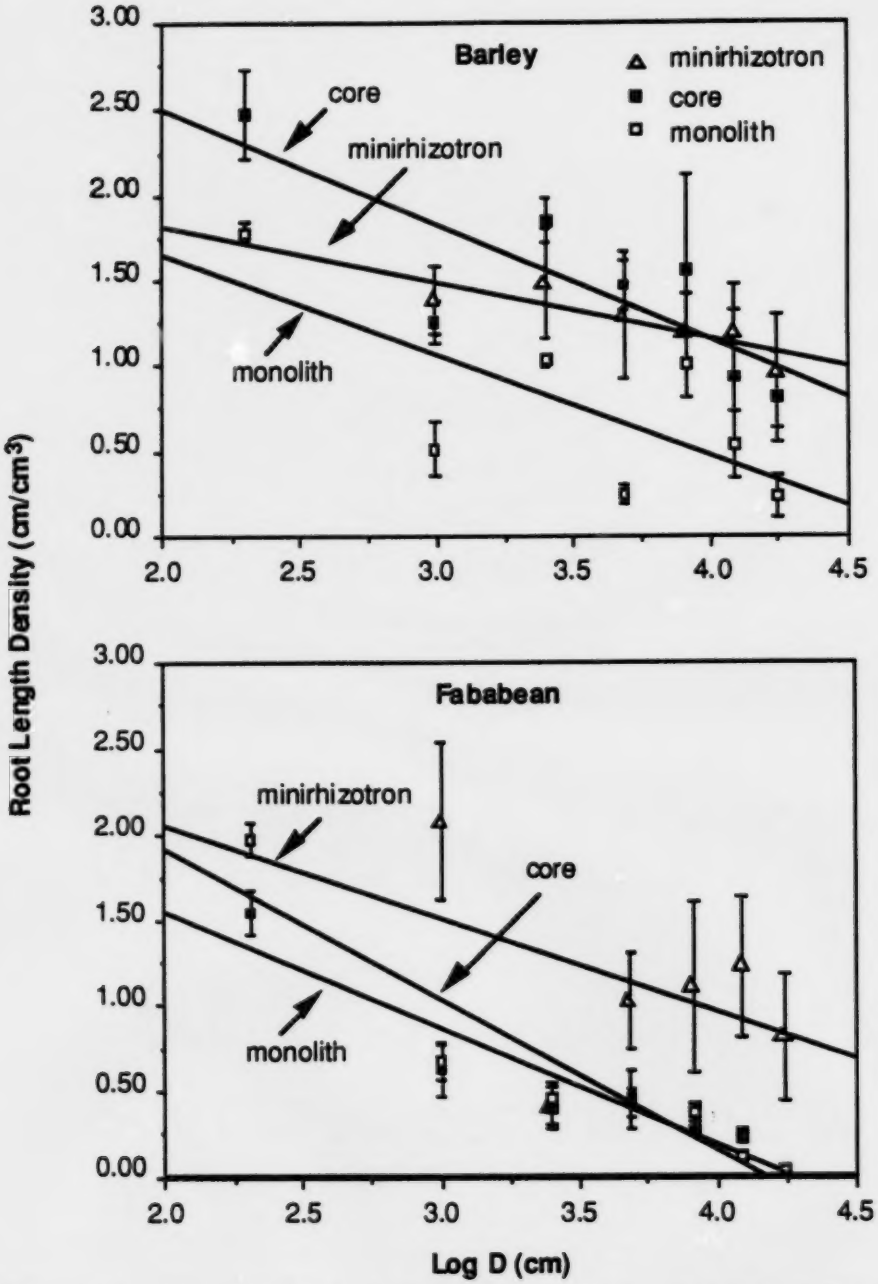

Figure II.4. Logarithmic depth distribution of root length density (RLD, cm cm^{-3}) for barley and fababean using minirhizotron, core and monolith methods. Vertical bars represent standard error of the mean. Each point is a mean of 3 replicates. Regression equations are given in Table 4.

the top 10 cm layer ranged between 0.12 - 0.16 mg cm^{-3} and 0.18 - 0.25 mg cm^{-3} for barley and fababean, respectively. Fababean exhibited a sharper decrease in root mass density with depth than barley. Estimates of RMD were not significantly different below 20 cm soil depth in fababean. In contrast, RMD was significantly different in the upper 40 cm depth for barley.

Logarithmic Root Distribution

Logarithmic curves were fitted to describe the relationship of RLD and RMD with depth for the two different crops (Fig. II.3). Equations describing these relationships are given in Table II.4. The regressions were first computed using means for all depths. The analyses were then repeated after removing one set of points. Root mass and root length density calculated using core and monolith methods decreased with depth for both crops. Correlation coefficients (r) between RMD and RLD and depth were generally higher for fababean than barley. Removal of data for the top 10 cm increased the correlation coefficient (r) between RLD and depth with the minirhizotron method. The r values for the minirhizotron method were significant in barley but nonsignificant in fababean.

Table II.4. Equations describing root mass (Y = RMD, mg cm^{-3}) and root length (Y = RLD, cm cm^{-3}) density† obtained by the different methods as a function of depth (D).

Crop	Method	Depth Interval (cm)	Equation‡	r§
Root Mass Density				
Barley	Core	0-70	Y = 0.24 (±0.06) - 0.04 (±0.02) Ln D	0.76*
	Monolith	0-70	Y = 0.19 (±0.06) - 0.04 (±0.01) Ln D	0.73*
Fababean	Core	0-70	Y = 0.32 (±0.04) - 0.07 (±0.01) Ln D	0.93**
	Monolith	0-70	Y = 0.46 (±0.08) - 0.11 (±0.02) Ln D	0.92**
Root Length Density				
Barley	Minirhizotron	0-70	Y = 0.54 (±0.66)+ 0.17 (±0.18) Ln D	0.38ns
		10-70	Y = 2.46 (±0.38) - 0.32 (±0.10) Ln D	0.85*
	Core	0-70	Y = 3.85 (±0.76) - 0.68 (±0.21) Ln D	0.82*
	Monolith	0-70	Y = 2.81 (±0.88) - 0.58 (±0.25) Ln D	0.73*
Fababean	Minirhizotron	0-70	Y = 1.35 (±1.20) - 0.08 (±0.34) Ln D	0.10ns
		10-70	Y = 3.13 (±1.98) - 0.54 (±0.52) Ln D	0.45ns
	Core	0-70	Y = 2.91 (±0.36) - 0.68 (±0.10) Ln D	0.95**
	Monolith	0-70	Y = 3.69 (±0.54) - 0.88 (±0.15) Ln D	0.93**

†Represents a mean of 3 measurements.

‡Numbers in parentheses indicate standard error of slope.

§Correlation coefficients are significant at: *, $p \leq 0.05$; **, $p \leq 0.01$; ns, not significant.

Regression Analyses

Linear regression analyses were used to describe the relationships for RMD and RLD between different methods (Table II.5).

Table II.5. Relationships between the minirhizotron (MR), core (C) and monolith (M) methods in estimating root mass (mg cm^{-3}) and root length (cm cm^{-3}) density† for barley and fababean.

Crop	Depth (cm)	Regression equation‡	r§
Root Mass Density			
Barley	0-70	M = -0.01 (±0.02) + 0.73 (±0.29) C	0.75*
Fababean	0-70	M = -0.02 (±0.01) + 1.49 (±0.10) C	0.99**
Root Length Density			
Barley	0-70	C = - 2.31 (±0.90) - 0.73 (±0.76) MR	0.39ns
	10-70	C = - 0.81 (±0.88) + 1.70 (±0.70) MR	0.77*
	0-70	M = 1.85 (±0.80) - 0.95 (±0.68) MR	0.53ns
	10-70	M = - 0.64 (±1.07) + 0.99 (±0.85) MR	0.51ns
	0-70	M = - 0.15 (±0.42) + 0.68 (±0.29) C	0.73*
Fababean	0-70	C = 0.53 (±0.50) - 0.01 (±0.42) MR	0.01ns
	10-70	C = 0.14 (±0.19) + 0.19 (±0.16) MR	0.52ns
	0-70	M = 0.66 (±0.66) - 0.08 (±0.56) MR	0.06ns
	10-70	M = 0.14 (±0.23) + 0.19 (±0.19) MR	0.46ns
	0-70	M = - 0.09 (±0.05) + 1.31 (±0.08) C	0.99**

†Represents a mean of 3 measurements.

‡Numbers in parentheses indicate standard error of slope.

§Correlation coefficients are significant at: *, $p \leq 0.05$; **, $p \leq 0.01$; ns, not significant.

Each depth represented paired values since CVs in individual observations were large (Tables II.1 and II.2). Mean RLD by the minirhizotron was excluded in the top 10 cm layer in the regression analyses because estimates of RLD were lower than the monolith method. In general a positive linear relationship existed between the methods. Correlation coefficients were significant ($p \leq 0.01$) between monolith and core methods in barley and fababean for both RMD and RLD.

Relationships between the minirhizotron and destructive methods resulted in considerably lower r values with an associated large SE of the slope. Intercepts obtained in barley were positive and negative in fababean. The RLD estimated by the minirhizotron and core methods were significantly ($p \leq 0.05$) correlated, but the RLD estimates for fababean were not significantly correlated.

Discussion

Root mass and root length distribution

de Willigen and van Noordwijk (1987) summarized root length densities at different growth stages in arable crops using different methods. Values of 4-5 cm cm^{-3} were found for barley and 0.5 - 2.0 cm cm^{-3} for beans in the 0-30 cm plowlayer. Our estimates of 0.42-2.08 in fababean are similar to those of de Willigen and van Noordwijk (1987) but estimates for barley (0.56 - 1.47) are considerably lower (0.56 - 1.47) (Figure II.1). This may be due to a higher decomposition rate of barley roots than fababean roots towards the end of the growing season. Xu and Juma (1992) concluded from their studies with different cultivars of barley that root lengths decrease faster than root mass toward the end of the season. They suggested that turnover of fine barley roots is rapid under field conditions.

High estimates of total root length and root length density in the entire rooting depth for barley confirms previous findings of Hamblin and Tennant (1987) that root length and root length density are greater in cereals than legumes. Although there were

differences in total root length between crops, total root mass was similar. This may be due to morphological differences in the root systems for the crops. Yamauchi et al. (1987) described the barley plant as having a "scattered type of root system" because almost all of the nodal roots ran obliquely in the profile. In contrast, fababean has a shallow or superficial root system with 40-48% of its root mass concentrated in the top 10 cm layer. Repeated growth of the primary root in fababean gives rise to a prominent tap-root that penetrates almost vertically downward. Crown roots are concentrated in the uppermost 10 cm layer and constitute a relatively high proportion of the total root mass (Weaver, 1926).

Comparison of the minirhizotron method with destructive methods

A logarithmic function adequately described root distribution patterns for the different methods. Madsen (1985) used this relationship previously for studying root distribution of spring barley in soils of different texture and under different climatic conditions. In contrast to the core and monolith methods, a significant relationship was only obtained when the data for the top 10 cm with the minirhizotron technique was not included. Underestimation in surface layers with the minirhizotron has been reported elsewhere (Cheng et al., 1990; Vos and Groenwold, 1987; Upchurch and Ritchie, 1983; Bragg et al., 1983; Gregory, 1979; Sanders and Brown, 1979). It is speculated that this may be partly due to inherent soil properties (Smucker et al., 1987), loss of resolution of roots growing horizontally near the surface (Beyrouty et al., 1987), effects of light leaks through gaps at the minirhizotron wall (Levan et al., 1987) and temperature differences at the glass soil interface (McMichael and Taylor, 1987). Vos and Groenwold (1987) also suggested that the physical presence of the tube may cause disturbance to the water regime above the tube thus reducing root length observations in dry periods compared with bulk soil.

An almost even distribution of root length with depth may have contributed to the significant linear relationship observed in barley between the minirhizotron and core method. In contrast, low rooting intensity for fababean in the subsurface soil layers may have reduced the probability of roots intersecting the sides of the tube and contributed to a positive intercept (Upchurch, 1987). Higher estimates of RLD with the minirhizotron compared to the monolith method at lower soil depths (>30cm) is more evident in fababean than barley. High variances from a low rooting intensity in the subsurface layers may have contributed to higher estimates of RLD hence affecting the slope of the equation. Gregory (1979) found a higher RLD with the minirhizotron in winter wheat and millet although root distribution patterns were similar with washed samples. Hansson and Andrén (1987) observed a higher relative abundance of roots in the subsoil compared to root biomass in barley, lucerne and meadow fescue. Vos and Groenwold (1987) also found similar results with fieldbean as reported here. Although minirhizotrons were carefully installed to maintain firm soil contact, preferential growth may have contributed to a higher RLD at the tube/soil interface than in bulk soil (Bragg et al., 1983; Upchurch and Ritchie, 1983).

Root morphology of fababean and soil physical properties are two possible reasons for enhancing preferential growth. Root branches in fababean do not spread obliquely as in barley but are oriented in a downward direction. Therefore, the roots will tend to pursue a course more or less parallel with the taproot. This is evident from the sharper decline of fababean RLD and RMD with depth as described by the logarithmic function. Secondly, preferential growth along the tube may have been further aggravated by shrinkage of soil during wetting and drying cycles where clay content for the surface horizons of soils in this area has been reported as exceeding 25% (Crown and Greenlee, 1978). Gaps at the wall of the minirhizotron have also been observed to promote preferential growth. Upchurch and Ritchie (1983) found that the presence of these gaps can lead to formation of root clusters or root growth along

the tube surface. Flexible tube walls have been proposed by many researchers to overcome this problem so that root growth at the tube-soil interface is more representative of bulk soil conditions (Gijsman et al., 1991; Box and Johnson, 1987; Maertens, 1987; Merrill et al., 1987).

Comparison of monolith and core methods

The correlation coefficients obtained between the monolith and core methods for root mass and root length densities were significant. The CV of root length and root mass of fababean using the monolith method was lower than the core method. The CV of root mass of barley using the monolith method was lower than the core method, however an opposite trend was found for root lengths. This suggests that the consistency of measurement using destructive sampling methods depends on type of crop. In barley, root length estimates by the monolith method were lower in the upper 40 cm soil depth compared to the core method but were higher at all depths in fababean. Hence, the monolith method was more consistent in fababean than barley. This is not surprising since the CV of root length for the whole profile obtained with the monolith method was higher in barley (42%) than fababean (24%). In comparison to fababean, barley roots do not exhibit a random variation in diameter but falls in discrete bands related to the order of the root. Hackett (1968) estimated that the fine branch roots or primary laterals (diameter 0.2-0.5 mm) can constitute up to 80 % of total root length in the root system of barley. Loss of these fine roots from washing a larger sample in the upper 40 cm soil layer may explain lower RLD estimates compared to minirhizotron and core methods. Also, incomplete separation of live roots from organic material, loss in detection of fine roots from overcrowding and the resolution limit of the video-image analyzer can introduce errors during automated root length measurements (Cunningham et al., 1989). These errors were much smaller in fababean than barley because of a

coarser and less dense root system. Vogt et. al. (1989) also concluded the monolith to be unsuitable for fine root data collection compared to the core method.

Coefficient of variation of root length and root mass amongst methods

In general, the high CVs in root length and mass for the methods are consistent with previous studies in the literature. van Noordwijk et al. (1985) summarized CV values of 30-50% in the 0-30 cm and 48% in the 30-60 cm depth for cereals using the core method. Sanders and Brown (1979) found values of 53 % and 14 % in soybean using core and scope methods and Belford and Henderson (1984) found CV ranging from 30-100% in wheat for both methods. The CV from minirhizotron observations has been reported as exceeding 100% and to decrease with increasing root intensity (Upchurch, 1987; Vos and Groenwold, 1987). In this study, the root length density was generally higher in barley and the CV was lower at all depths than fababean. The large CV observed in fababean with the minirhizotron method requires that more observations be taken relative to the other methods. In addition, this large variation explains the observed low correlation coefficients and the need for statistical transformation of root data (Upchurch, 1987).

Root distribution patterns is affected by soil (e.g structure) and plant factors (e.g branching) which can contribute to differences in variation between methods (van Noordwijk et al., 1985). This study shows that CVs differences may also be related to soil sampling volumes. A larger soil volume extracted by monolith compared with core and minirhizotron may have contributed in reducing root spatial heterogeneity and hence the CV.

Implications

Root distribution can be quantified using destructive and non-destructive sampling methods. The results from this study has shown that the core and monolith methods can be used for different crops. However, the minirhizotron technique still needs to be

calibrated for different methods especially in the top 10 cm layer. Destructive sampling still remains the method to quantify root growth in this layer.

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Chapter 3. A Color Composite Technique for Detecting Root Dynamics of Barley from Minirhizotron Images

Introduction

Fine branch roots or primary laterals (0.2-0.5 mm diameter) can constitute up to 80% of the total root length in a barley root system (Hackett, 1968). The turnover of these roots represents an important source of carbon into the soil ecosystem (Smucker, 1984). At present, little is known about soil microsites and their effects on root dynamics (Vogt and Bloomfield, 1991), since attempts to develop new techniques have not been successful (Persson, 1990). Destructive sampling, by coring over the growing season, overestimates root dynamics because spatial heterogeneity of roots results in high variances (Singh et al., 1984). Core sampling also requires separation of live from dead roots, removal of organic debris and recovery of fine roots which can be a formidable problem (Russell, 1977). Radioactive tracer techniques, such as the use of ^{14}C dilution, avoids the need for separation of roots but relies on complete translocation of the tracer prior to first sampling (Caldwell and Camp, 1974). Non-destructive staining techniques to study root dynamics are less tedious and labour intensive than core methods, but require a porous media for complete dye infiltration (Carman, 1982) .

In situ non-destructive techniques have also been used to quantify root dynamics. Head (1966) used hand tracing with different colors of wax pencils on a rhizotron window to indicate changes in root length. Huck and Taylor (1982) measured dynamics at the Auburn rhizotron by marking lengths of individual roots and tagging them with an identifying number. Although qualitative and quantitative data were generated on root senescence and turnover dynamics, the method was described as monotonous and not suitable for measurements of fine roots (Huck and Taylor, 1982). The rhizotron is also an expensive facility and microclimatic conditions can be

significantly different from adjacent field plots (Huck and Taylor, 1982; Taylor et al., 1990).

The minirhizotron technique involves installing clear acrylic or glass tubes into the soil and inserting a device (fiber optics or video camera) so that roots can be observed and video-recorded at the soil-tube interface. This is an *in situ*, non-destructive technique which minimizes point-to-point variation by allowing repeated measurements at the same depth over time (Taylor, 1987). van Noordwijk (1987) monitored appearance and disappearance of roots by overlaying sequential photographs from minirhizotron observations using counting grids. Cheng et al. (1990) quantified root dynamics by hand tracing roots observed on a TV monitor onto a plastic sheet that covered the screen. Both researchers agreed that more efficient means are required for root data quantification.

Digital image processing has permitted a permanent, digital record of video images to be obtained, analyzed, and interpreted with a personal computer and specific software. Lussenhop et al. (1991) has stated that "the coupling of video images from minirhizotron observations with image analysis offers the exciting prospect of reducing the labour of data analysis, increasing accuracy, and allowing direct measurements of rates and turnover". Smucker et al. (1987) used image analysis to measure root length, width, and root surface area from minirhizotron images. However, work in quantifying root growth dynamics by image analysis is scarce (Lussenhop et al., 1991) and there is a present need for replacing the monotony of hand tracer methods with computerized techniques (van Noordwijk., 1987; Cheng et al., 1990).

A fundamental assumption in detecting a change using digital images is that there exists a difference in the spectral response of a pixel on two dates (Jensen, 1986). The image overlay technique, which is akin to image differencing, has proven useful in remote sensing to monitor temporal changes in land cover and land use. This technique has been successfully used in detecting scene changes by assigning separate colors to

images on different dates (Banner and Lynham, 1981; Crapper and Hynson, 1983; Howarth and Boasson,1983; Hall et al., 1984; Kirchhof et al., 1984). Application of this technique using computer-assisted image analysis techniques offers the power for analyzing sequential minirhizotron observations.

The objective of this paper was to adapt this technique for, qualitatively and quantitatively, measuring the dynamics of barley (*Hordeum vulgare* L. cv. Bonanza) roots at tillering, stem extension, heading, dough and ripening growth stages *in situ* .

Materials and Methods

Growth Containers and Minirhizotron Installation

Three boxes (80 cm long x 80 cm wide x 75 cm deep) were constructed from plywood (19 mm thick) for the purpose of this study. Growth media consisted of sieved soil material from the Ap horizon of an Orthic Black Chernozem (Typic Cryoboroll). This soil is naturally endowed with a thick mollic epipedon which has granular structure, high nutrient and base status, and neutral pH (Crown and Greenlee, 1978). Each container was compacted uniformly to a depth of 70 cm at a bulk density of 1.2 Mg m^{-3}. Urea (50 kg N ha^{-1}) and triple superphosphate (20 kg P_2O_5 ha^{-1}) fertilizer were broadcasted and incorporated in the top 10 cm layer. Barley seeds were sown in a hexagonal arrangement at a planting density of 204 plants m^{-2}. The plants were grown at 21 °C in a greenhouse at Edmonton (53 °N, 113 °W) without artificial lighting in June, July and August 1990. The daylength was between 15 to 16 hours. The 30-year (1951-1980) average monthly radiation during June, July and August ranges between 18 to 22 MJ m^{-2} depending on cloud cover (Atmospheric Environmental Service, 1982). Growth stages were identified using keys of Zadoks et al. (1974).

To quantify barley root dynamics, minirhizotrons (tubes measuring 58 mm diameter, 2 mm thickness and 120 cm length) with permanently sealed bottoms were

specially constructed from glass. Transparent plastic grid strips (1 cm x 1 cm) were mounted along the sides of the tubes which were then installed at 45° angle in the center of the box prior to soil filling. The top 100 mm of tube which projected outward from the face of the box was wrapped with black electrical tape. This was done to prevent light from entering the rooting zone which can have inhibitory effects on root growth rate (Furuya and Torrey, 1964). When observations were not taken, the tubes were capped with rubber stoppers to further block light, minimize moisture condensation and insulate against temperature gradients.

Video Images Acquisition

The video-recording system used in this study consisted of a Circon color video camera (MV 9011), a 12.7 cm color video monitor (a Sony color TV with a 38 mm tube) and a Sony Video Cassette Recorder (VCR) model EVO-210 (Circon Corporation, Santa Barbara, CA). Roots in each 2.4 cm^2 window (2 cm horizontal x 1.2 cm vertical) were displayed on the monitor by inserting the micro video camera down the minirhizotron. The camera was moved at a rate of 2-3 mm sec^{-1} to allow ample time for focusing and stationary viewing. The lines of the grid were used as a guide for camera repositioning on objects within the field of view. All images were video-recorded and stored on 8 mm Sony tape (P6-90MP) using the EVO-210 recorder at a tape speed of 1.43 cm sec^{-1} (SP). Minirhizotrons were observed at the following plant growth stages: Zadoks' code 15 (Date1, June 30), Zadoks' code 39 (Date 2, July 15), Zadoks' code 56 (Date 3, July 20), Zadoks' code 87 (Date 4, August 4), Zadoks' code 92 (Date 5, August 18). These growth stages corresponds to tillering, stem extension, heading, dough and ripening events of crop ontogeny.

Digital Image Processing

Image Analysis System

The hardware of the image analysis system consisted of a Powermate 386/25 MHz Personal Computer (PC) equipped with a video frame grabber-digitizer board (Intel 80387-25) math co-processor, VGA-compatible video graphics board (VGB), 1024 K RAM, 100-MB hard disk and a Microsoft mouse. An IBM Proprinter II was used for logging and statistical tracking on various digital data computations. A MultiSync 2A color monitor displayed menus and control information on the operational software. Images stored on 8 mm tape were replayed on the EVO-210 VCR until a preferable depth was obtained with roots. The video signal was directed to the digitizer producing an 8 bit, 512 horizontal x 512 vertical digital image with 262,144 picture elements or pixels (Nichols et al. 1983). A pixel value of 0 is designated as black and 255 as pure white. Lookup tables (LUTs) and graphics capabilities of the computer enables display of unprocessed and processed digital images in real-time on an Electrohome ECM 1301 high resolution analog RGB (Red, Green, Blue) monitor.

Software User Interface

All digital image processing of minirhizotron recorded data was accomplished in a DOS environment using RS12 Image Analysis Software (V3.0) developed by Eidetic Digital Imaging Ltd. (Brentwood Bay, British Columbia). This software is both menu driven and interactive which allows for a wide variety of standard image processing techniques to be performed on digital data. Various subroutine programs such as image restoration, registration, enhancement and classification can be used to manipulate digital data (Peet, 1990).

Analog/Digital Conversion

Analog images corresponding to tillering (scene **a**), stem extension (scene **b**), heading (scene **c**), dough (scene **d**) and ripening (scene **e**) growth stages were digitized

from 8 mm video tape recordings. The tapes on the EVO-210 VCR were replayed and all images throughout the 70 cm depth interval were scanned. Selection of a particular depth was made on the basis of clarity of images, reference points, and presence of roots in the center of displayed area.

Images Registration

The first step in the image registration process was the selection of reference points. These were manually obtained by pattern recognition of the images to be registered. Scene **a** was selected as the master image (base image) and was compared in a specified searching area to the slave image window (image to be registered, scenes **b, c, d,** and **e**). The comparison, or matching criteria of the windows, was carried out based on the approximate location of recognizable reference points in the master and slave windows. A total of 8 reference points with respective x, y coordinates were used over the entire image in the transformation process. The reference points were subjected to a least squares regression analysis to generate mapping polynomials relating scenes **b, c, d** and **e** with scene **a**. This process was repeated by removing and/or adding reference points to obtain mapping polynomials with the smallest residual error. The original coordinates, the computed x and y coordinates, and the residuals were displayed on the computer screen and printed on the lineprinter. A first order affine transformation was used to map scenes **b, c, d** and **e** to scene **a**. Once the threshold residual error was obtained in the transformation process, a cubic convolution algorithm was used to resample the images since it provides a sharper image and avoids the disjointed appearance of images resulting from the nearest neighbour method (Lillesand and Kiefer, 1987). This process is referred to as intensity interpolation since pixel brightness values (BVs) from the slave image will not usually project to exact pixel locations in the master image (Richards, 1986).

Digital Image Classification

Specific digital ranges corresponding to two information classes, roots versus matrix, were determined from the resampled images by pixel readout and histogram analysis. Brightness values for roots were generally greater than 65 with the soil matrix having values less than 30. A color density slice was applied to the images once digital ranges were established with the colors red and blue assigned to root and soil matrix, respectively. Small areas (≤30 pixels) in the resulting image with similar BVs as roots were merged into the soil matrix using a small region conversion subroutine program. The resulting output was a classified image on the display monitor with accompanying hardcopy data on the number of pixels in each information class. These values were used to calculate relative percentage areas occupied by root and soil matrix respectively within 2.4 cm^2 viewing area.

Visual Image Analysis

A linear contrast stretch was performed on registered images to enhance the video output signal display and their visual presentation. Since cubic convolution changes pixel values during intensity interpolation (Richards, 1986), histogram analysis and pixel readout were performed to determine upper and lower gray limits for contrast stretch enhancement. By linearly stretching the data, digital values in the lower end of the original histogram are assigned to extreme black (0) and a high end is assigned to extreme white (255). The remaining pixel values are distributed linearly between these extremes (Lillesand and Kiefer, 1987).

The enhanced contrast stretch images were superimposed 2 and 3 at a time to make (red, green) and (red, green, blue) color composites via the red, green and blue colors of the image display. These colors are referred to as additive primaries which can combine to produce other complementary colors (Lillesand and Kiefer, 1987). Combinations of red-green-blue, red-green, blue-red and blue-green can give white, yellow, magenta and cyan colors respectively (Lillesand and Kiefer, 1987). By

combining the three primary colors, areas of no change will appear white whereas areas that had changed will be highlighted in one of the primary or complimentary colors (Table III-1) In this way, one can detect temporal changes in a particular scene (Banner and Lynham, 1981; Howarth and Boasson, 1983). All color composites were photographed from the image display monitor on low speed Ektachrome film (ASA 100).

Table III-1. Illustration of change detection by assigning red, green and blue colors to individual images on three (3) dates

	Change Detection		
Observed Color	Red = Date 1	Green = Date 2	Blue = Date 3
Red	†+	–	–
Green	–	+	–
Blue	–	–	+
White	+	+	+
Yellow	+	+	–
Magenta	+	–	+
Cyan	–	+	+

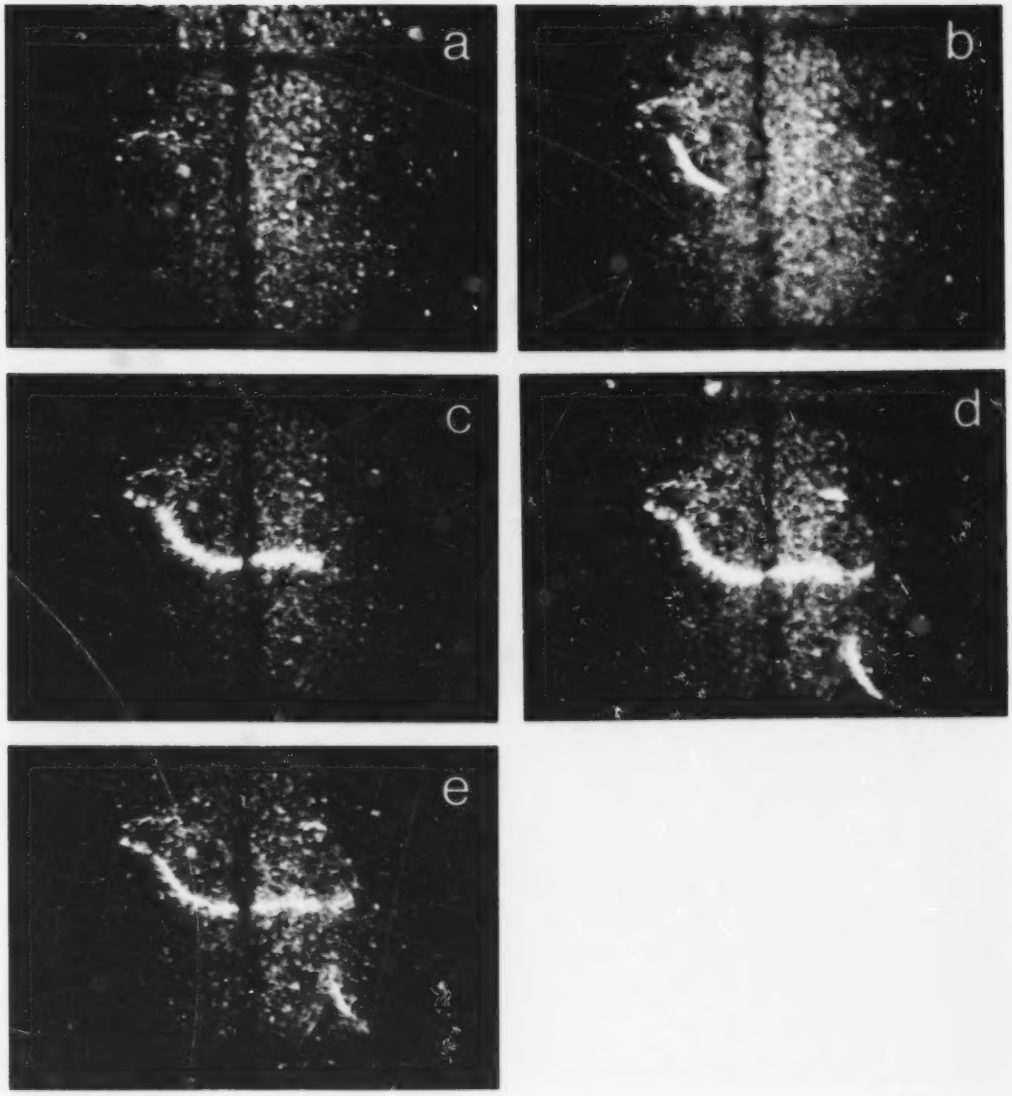
†(+) indicates present and (–) indicates absent of object(s) on particular dates.

Results and Observations

Digitized Images

Lighting inconsistencies from non-uniform tubing were observed at the periphery of some digitized images. Manual adjustment of the brightness and contrast controls on the RGB monitor provided a better visual interpretation of the digitized images. Digitized images at tillering (scene **a**), stem extension (scene **b**), heading (scene **c**), dough (scene **d**) and ripening (scene **e**) growth stages are displayed in Plate III-1. Scene **a** shows an absence of roots. However, objects of similiar shades of gray as roots are displayed in this scene. It is unclear whether these objects are quartz grains, other soil particles, root tips or soil fauna.

Plate III-1. Original unregistered digitized images (**a to e**) at a depth of 66 cm within 2.4 cm^2 minirhizotron viewing area. The scenes correspond to (**a**) tillering, (**b**) stem extension, (**c**) heading, (**d**) dough, and (**e**) ripening growth stages in barley. Gridlines appear darkly toned in the images.


a
b
c
d
e

A white root tip appears in scene **b** and becomes extended in scene **c** by intersecting the grid line. Root hairs are observed in scene **c** at some distance from the root tip. New root growth was observed by the appearance of several root tips in scene **d**. Scene **e** shows roots which have partially decomposed.

Quantitative Root Dynamics

Application of density slices to the original digitized images resulted in separation of roots from the soil background. Classified color images depicting two information classes, root and soil matrix, for specific growth stages are displayed in Plate III-2. Roots are delineated in red against a blue soil matrix. The number of pixels delineated in each information class from density slicing was used to calculate percentage relative areas occupied by roots and the soil matrix within 2.4 cm^2 viewing area. Root growth dynamics for barley is illustrated in Fig. III-1.

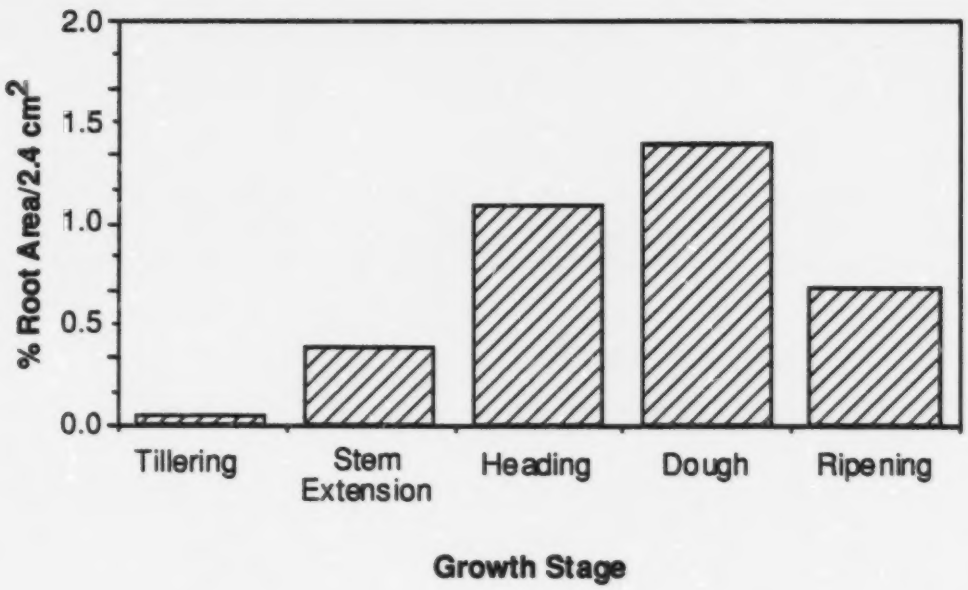
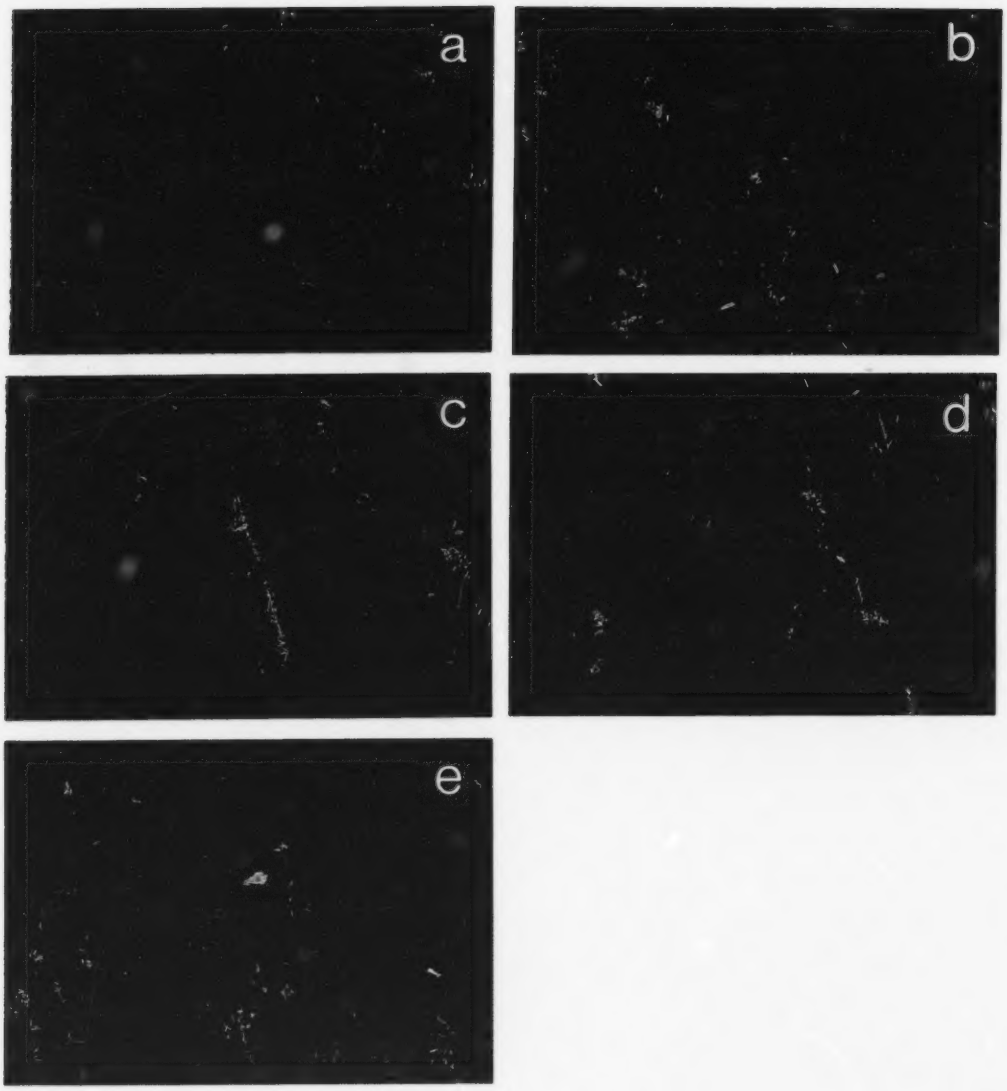


Figure III-1. Temporal dynamics of barley roots within a 2.4 cm^2 area obtained at a depth of 66 cm.

Plate III-2. Classified single images (**a to e**) at a depth of 66 cm depicting root (red) and soil matrix (blue) after a density slice is applied to individual growth stages. The scenes correspond to (**a**) tillering, (**b**) stem extension, (**c**) heading, (**d**) dough, and (**e**) ripening. Red indicates a pixel brightness value greater than 65 and blue a value less than 30.


a
b
c
d
e

In general, percentage areas occupied by roots ranged from 0.03 to 1.39 of the viewing surface area. An increase in root growth was observed from tillering (0.03) to dough (1.39) growth stages followed by a sharp decrease at ripening (0.68) (Fig. III-1). Changes in root growth from tillering to dough were positive with a maximum value obtained at heading. Thereafter, incremental changes declined reaching a negative value at the ripening growth stage.

Qualitative Root Dynamics

A considerable shifting in control points and roots were observed during transformations. This was most evident in scene e. Registration was achieved in all images within an accuracy range of 0.04 to 1.03 mean pixel residual error. Temporal root dynamics were observed from each color composite by assigning two colors (red, green) and three colors (red, green, blue) to specific growth stages (Plates III-3 and III-4). Interpretative keys for the two plates are presented in Tables III-2 and III-3, respectively.

Table III-2. Interpretative key for Plate III-3.

Image		Color Assignments	
Original	Contrast Stretch	Red	Green
a	e	Tillering	Stem Extension
b	f	Stem Extension	Heading
c	g	Heading	Dough
d	h	Dough	Ripening

Table III-3. Interpretative key for Plate III-4.

Image		Color Assignments		
Original	Contrast Stretch	Red	Green	Blue
a	d	Tillering	Stem Extension	Heading
b	e	Stem Extension	Heading	Dough
c	f	Heading	Dough	Ripening

Plate III-3. Color composite at a depth of 66 cm after mixing the red and green primary colors of the image display. Scenes **a-d** and **e-h** indicates color composite before and after contrast stretch respectively. An interpretative key for this plate is given in Table III-2.

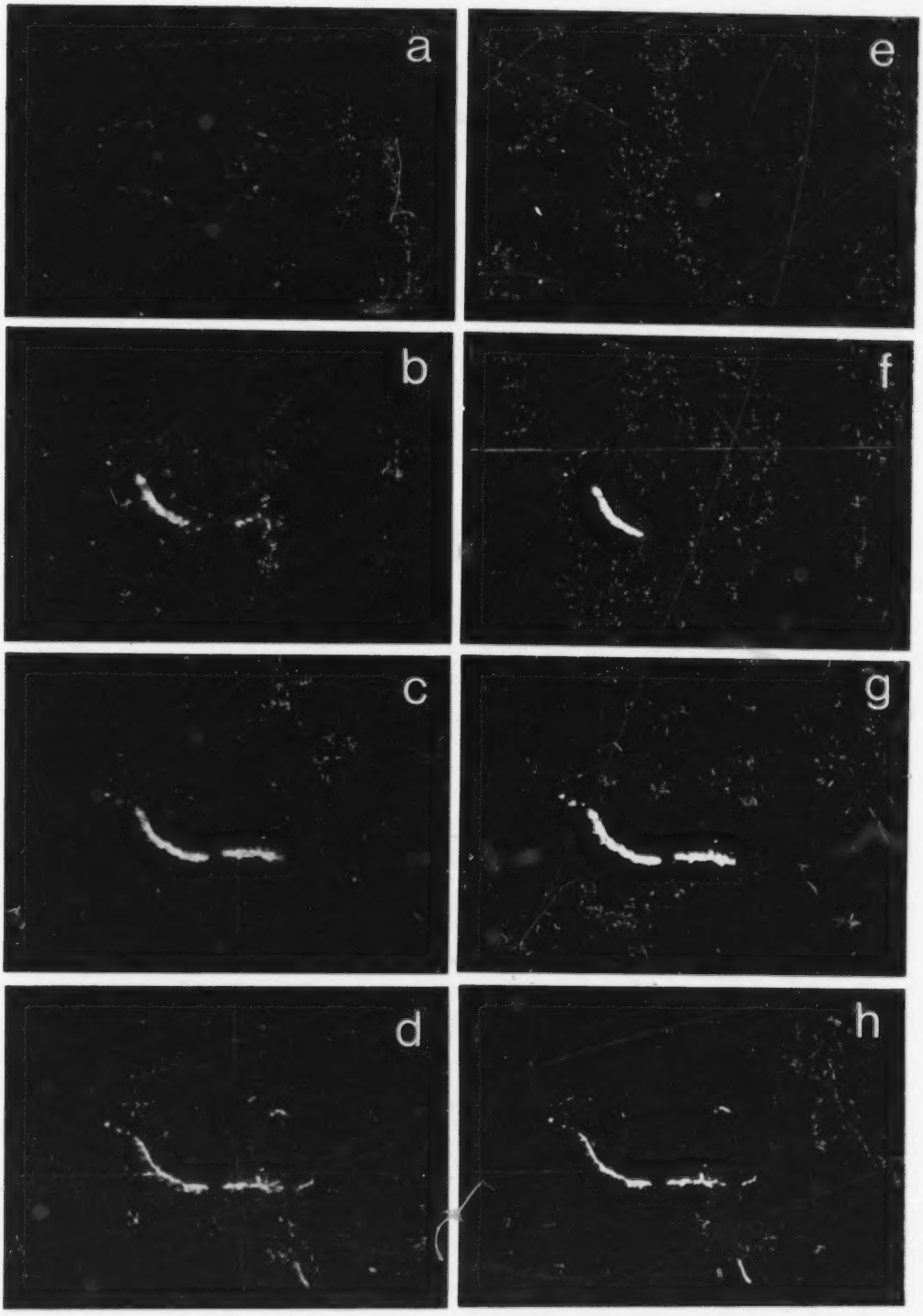
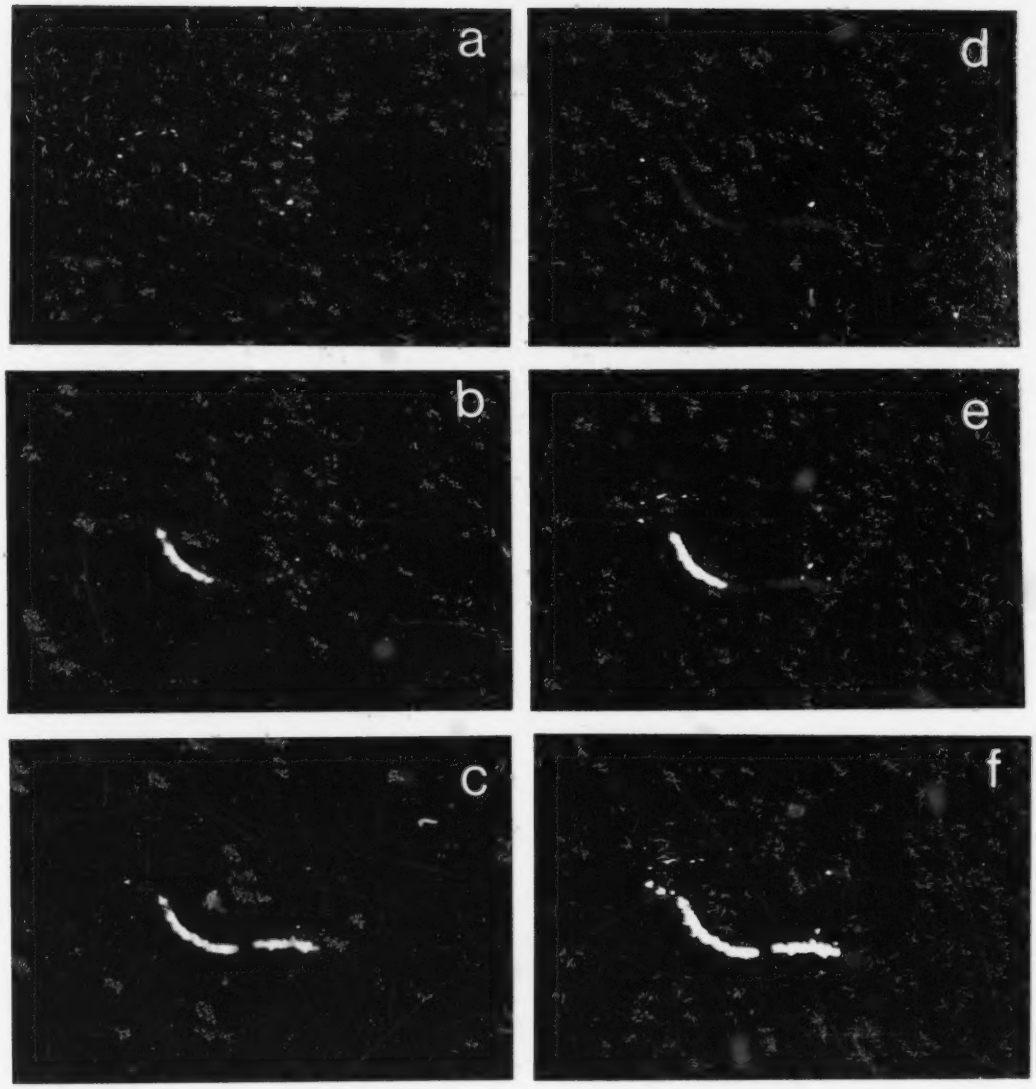

a
e
b
f
c
g
d
h

Plate III-4. Color composite at a depth of 66 cm after mixing the red, green and blue primary colors of the image display. Scenes **a-c** and **d-f** indicates color composite before and after contrast stretch, respectively. An interpretative key for this plate is given in Table III-3.


a
d
b
e
c
f

A continuous yellow tone in composite **3a, 3b, 3c** and white tone in composite **4a, 4b** suggests an accurate overlay in the transformed images. However, composites **3d** and **4c** appears greenish and bluish from distortion in roots with associated background objects. This suggests poor registration in the images. Green is separated from red (**3d**) and blue from red and green (**4c**). Distortion is more evident on the left side as compared to the center and the right side of the registered images. Enhancement of the transformed images by contrast stretch increased color brightness and more clearly demonstrated change detection in the registered images (**3e to 3h** and **4d to 4f**).

Transition in root growth dynamics is observed from the two color composite. An increase in root growth during stem extension, heading and dough growth stages is observed as an increase in area of green color (**3e, 3f, 3g**). Root decomposition during ripening is observed in red (**3h**). A yellow color indicates root material present on 2 consecutive dates (**3f, 3g, 3h**). Similiar interpretational patterns on growth dynamics can be extracted from the 3 color composites. No change in root area is observed in white (**4e, 4f**) from overlaying 3 scenes and as yellow, cyan and magenta from an overlay of 2 scenes. Red, green and blue primary colors indicates areas of change. Root elongation during heading and dough growth stages is observed as an increase in blue color (**4d, 4e**). Amount of root decomposition during heading and dough growth stages is observed in yellow (**4f**).

Discussion

Video-Recorded Images

Image quality is an important prerequisite for successful analysis of video-recorded data (Smucker et al., 1987) since it controls accuracy of information that can be extracted (Vleck and Cheung, 1984). High quality analog images obtained in this study may be attributed to the resolution of the microvideo camera and the VCR (Vlcek and Cheung, 1984), and the installation and optical quality of minirhizotrons (Brown

and Upchurch, 1987). Low battery power can also contribute to photometric distortion from poor illumination of incandescent lights surrounding the camera. Bernstein et al. (1983) also showed that analog-to-digital conversion can cause quantitative and signal errors in digitized images. Non-uniform diffusion of light from tube curvature (Brown and Upchurch, 1987) may also result in inconsistencies in brightness observed in the digitized images. Square tubes have been proposed as a means of providing flatter surfaces for camera focussing and maintaining uniform lighting over the viewing area (Brown and Upchurch, 1987).

Geometric Distortion

Geometrically distorted images can arise from non-perpendicular viewing, camera tube non-linearity or other transmission artifacts. It was evident from the non-aligned images in the video-recordings that using the relatively coarse grid lines on the minirhizotron provided little opportunity for correcting camera repositioning. This can result in a loss in resolution since video image analysis requires a stationary image to be present as one video field (Vleck and Cheung, 1984). Geometric distortion can be minimized by using the modified microvideo camera system with a registration handle for quantifying spatial and temporal root dynamics (Ferguson and Smucker, 1989). With their system, positioning of camera was controlled by manually withdrawing the camera along the minirhizotron tube at 1.2 cm intervals while images are recorded for 3 seconds at each position (Ferguson and Smucker, 1989).

Qualitative Root Dynamics

Detection of temporal scene changes relies on an accurate image-image registration of digital data. Jensen (1986) suggested that registration should be within 0.25 to 0.50 pixels to each other so as to avoid spurious areas of change between two data sets. Bernstein et al. (1983) indicated that 16 reference points should be used for

achieving an acceptable degree of accuracy assuming that reference points are located within 0.33 pixel accuracy or better. In this study, a maximum of 8 reference points were visible in the master and slave images and the residual error during registration of images was 1.03 pixels or less. A number of factors may have contributed to a higher residual error. Firstly, selection of reference points consisting of a set of common features present in both master and slave images was often a subjective process (Bernstein et al., 1983). This process was time consuming and frustrating in some cases because reference points with similar BVs may represent completely different objects. Additionally, distinctive features or color characteristics of quartz grains, other soil particles, root tips and soil fauna were not easily discernible which made separation difficult. Analog images are qualitative whereas digital images are quantitative. Once the analog video images are digitized with a black and white system the color differentiating characteristic is removed. A compounding of errors in locating similiar reference points in master and slave images may have contributed to registration inaccuracies. Secondly, displacement of roots and movement of reference points due to movement of soil material was an observed phenomena during selection of reference points. This was a more significant problem in the last growth stage (scene e) than in the earlier ones. Movement of material may be attributed to settling of soil in the growth containers with time (Böhm, 1979), diurnal variations in root diameter from moisture fluctuations (Huck et al., 1970) and/or reorientation of soil particles from shearing during root elongation (Goss, 1991). Although minirhizotrons were firmly installed into proper position, the possibility of tube movement cannot be ignored. Root and soil material which shifted in the scene limited the number and distribution of reference points and subsequently registration of images. This may have contributed to mis-registration of scene 5 with scene 4. A number of algorithms have been developed to automatically locate reference points in master and slave images (Bernstein et al., 1983) but were not available in our image analysis software.

Quantitative Root Dynamics

Discrimination of roots from the soil matrix by density slicing at different growth stages gave direct estimates of growth dynamics within the viewing area (2.4 cm^2). Thresholding often resulted in an inclusion of background objects with similiar BVs as roots (>65). Inclusion areas usually ranged from 20-30 pixels. Delineation of roots from the soil matrix was often a subjective and interactive process. The absence of a smooth root boundary often resulted in difficulty in establishing threshold limits. This problem was further augmented by the attachment of soil particles to roots and discoloration of roots during senescence. While the system can be used to discriminate with certainty between BVs, subjectivity by the operator leads to questions whether information gained by this procedure is valid. Assigning specific digital ranges to classes of information is dependent on an operator's judgement following a set of personal criteria which may vary between individuals. Therefore, inaccuracy in quantitative estimates of root dynamics may be due to human intervention. This is necessary to counter balance computer logic.

Root growth increased from tillering and reached maximum elongation at heading. This was followed by the decomposition of root cortex during dough and ripening growth stages. Decomposition of root material amounted to 50% during dough and ripening which is qualitatively supported from the color composites. Xu and Juma (1992) concluded that root length decreased more than root mass at heading and ripening growth stages in different barley cultivars under field conditions. This was attributed to decomposition of fine roots towards the end of the growing season. The dynamics of these fine branch lateral roots therefore represents an important source of organic substrates for soil heterotrophs.

Implications

Subtle changes in root growth and decomposition can be detected *in situ* using the technique of color composites. This technique has a potential for directly estimating root turnover rates in different crops while reducing the labour and processing time involved in destructive core sampling. However, a fundamental consideration of this technique is that pixels at the same image-matrix location for two or more images represents an identical soil location. Accurate registration of digital images is therefore vital for detecting changes between scenes. Subjectivity in discriminating between similar reference points and movement of soil material can contribute to inaccurate registration. In addition, the settling of soil in the growth containers over time may cause a relocation of reference points. This may not be a problem under field conditions because of higher bulk densities. The use of an elaborate system of reference markers or pointers on the tube may be a means of overcoming registration fatigue whilst increasing accuracy. In this way, the images are registered with respect to fixed control points rather than a dynamic soil matrix.

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Chapter 4. *In Situ* Detection of Root Dynamics of Barley and Fababean Using a Color Composite Technique

Introduction

Interest in root demography arises from efforts to improve estimates of root production, root turnover, and the proportion, or cost, of photosynthate needed to grow and replace roots (Fogel, 1991). Experimental approaches to quantify root turnover rates by destructive sampling are confounded by spatial and temporal variability of roots (Atkinson, 1985; de Willigen and van Noordwijk, 1987), rapid turnover of fine roots and laborious, tedious and time-consuming process of separating roots from soil (Russell, 1977). For these reasons, root turnover tends to be poorly estimated (Persson, 1990; Singh et al., 1984).

Direct measurements of root decomposition have often been recorded from roots previously separated from the soil and then buried (Herman et al., 1977; Malone and Reichle, 1973; Waid, 1974; Dickinson, 1982). However, the isolation of roots from their natural setting does not provide information on the actual rates of decomposition in soil. In the last 20 years, there has been an evolution of non-destructive methods to study roots *in situ*. At present, however, there is limited information on longevity of fine roots (Fitter, 1985; Lussenhop, 1990) because attempts to develop new techniques permitting continuous recording have not been successful (Persson, 1990).

Rhizotrons have been used to study growth and decomposition of individual roots. However, rhizotron installation and maintainance costs are large (Taylor et al., 1990) and there can be difficulties in making measurements of fine roots (Dickinson, 1982). Furthermore, root dynamics observed from the rhizotron window have to be extrapolated with caution to field conditions (Vogt and Bloomfield, 1991). The recently improved minirhizotron technique (Taylor, 1987) is a rapid, non-destuctive

technique which holds great potential for temporal monitoring of fine root dynamics *in situ* (Lussenhop et al., 1991). The rate of root appearance and disapperance and changes in root lengths can be measured by a visual inspection of a series of photographs from the same minirhizotron depth over time (de Willigen and van Noordwijk, 1987). The color composite technique adapted in Chapter 3 was used to detect changes in growth and decomposition of fine roots of barley from minirhizotron images and may be an effective tool for determining root turnover rates in different crops. Therefore, the objectives in this study were to use the minirhizotron technique to: (1) monitor root length intensity for barley and fababean crops at specified growth stages over the growing season; (2) use the color composite technique for a visual interpretation of root dynamics *in situ*; and (3) quantify root length turnover rates for individual barley and fababean roots.

Materials and Methods

Boxes, Growth Media and Minirhizotron Installation

A completely randomized block experimental design (2 crops x 3 replications) was used for monitoring barley and fababean root dynamics over the season of growth. Six boxes of dimensions 80 cm x 80 cm x 75 cm were constructed from plyboard (19 mm thick). Details of growth media, installation of minirhizotrons and plant growth conditions are described in Chapter 2.

Video Images Acquisition

Plant roots intersecting the minirhizotron tube were observed with a Circon color video camera (MV 9011) connected to a 12.7 cm color video monitor (a Sony color TV with a 38 mm tube) and a 8 mm Sony video cassette recorder (VCR) model EVO-210 (Circon Corporation, Santa Barbara, CA). Images produced by the camera were observed on the monitor and video-recorded on a 8-mm tape simultaneously, as

described in Chapter 3. Minirhizotrons were observed and video-recorded at specific growth stages in barley and fababean as shown in Table IV.1.

Table. IV.1 Minirhizotron observation date and growth description of barley and fababean

Observation Date	Growth Code	Description of growth stage†
Barley		
30 June	15	Tillering (1-2 tillers present)
15 July	39	Stem Elongation (flag leaf ligule just visible)
20 July	56	Heading (3/4 of inflorescence emerged)
4 August	87	Dough (hard)
18 August	92	Ripening (caryopsis extremely hard)
Fababean		
30 June	35	Leaf (8 leaves unfolded)
15 July	53	Bud formation (first flower racemes visible at shoot tip)
20 July	63	Flowering (>3 flower racemes/plant in bloom)
4 August	74	Pods visible in the middle inflorescence
18 August	78	Early ripening of pods (green ripeness)
1 September	87	Late ripening (2/3 of all pods darkly colored)

†Following Zadoks and EPPO code for barley and fababean, respectively.

Analyses of Video Images

Archived video images on 8-mm tapes (2 cm horizontal x 1.2 cm vertical) were retrieved and analyzed for root intersects (N) using the counting techniques of Upchurch (1987) and were summed to 10 cm depth intervals. Total root lengths (RL, cm) at the glass surface were determined using the line intersection method (Newman, 1966): $RL = \pi NA/2H$, where N = number of intersects for 10 cm depth, A = area of

the observation (cm^2) and H = total length of straight lines (cm). A root length intensity (RLI, cm cm^{-2}) was derived from the equation: RLI = RL/A.

Color composites using the red and green color channels of the images display were used to detect changes in root growth and assumed decomposition for the two crops. Analog images recorded at different growth stages and from the same soil depth were digitized from 8-mm tapes. The details on the image capture and analysis system were described in Chapter 3. A depth of the minirhizotron was selected based on the criteria of image clarity, noticeable reference points and the presence of a single root in the displayed central area. To illustrate temporal root dynamics, master and slave images corresponding to specific growth stages were registered. Images were registered using a first order affine transformation algorithm then resampled by a cubic convolution intensity interpolation. Registration accuracies were obtained within the range of 0.60 to 1.45 pixels for fababean and 0.25 to 1.85 pixels for barley, respectively. A total of 8 control points were used over the entire images. Transformed images were enhanced by linear contrast stretch then superimposed two at a time to create color composites via the red and green color channels of the image processor. All color composites from the image display monitor were photographed on low speed Ectachrome color film (ASA 100).

Specific digital ranges corresponding to two information classes, roots versus matrix, were determined from the resampled images by pixel readout and histogram analysis. A color density slice (red, blue) was applied to the images once digital ranges were established followed by small region conversion. A combination of these two procedures generated the number of pixels occupied by root and soil matrix within 2.4 cm^2 viewing area as described in Chapter 3. These values were used to calculate relative areas occupied by root and soil matrix at each growth stage. The turnover of root length (T_l) was calculated from density slice areas over the season of growth using the equation : $T_l = L_d\ (e)/L_n\ (e)$ where $L_d\ (e)$ = cummulative length of decomposed or

disappeared roots at the end of the growing season and L_n (e) = cummulative length of new roots since the start of the growing season (de Willigen and van Noordwijk, 1987).

Root Analyses

A pinboard (75 cm x 70 cm x 8 cm) was used to extract a soil monolith at the ripening growth stages to quantify specific root length (SRL, m g^{-1}) and root diameters for the two crops. The procedures for monolith extraction, root washing and sorting and root length measurements are described in Chapter 2.

Dried root samples were ground to <1.3 mm prior to chemical analyses. Total C was measured by dry combustion in a LECO carbon automatic analyzer (Leco CR-12 Carbon Systems 981-600, LECO Corp., St. Joseph, MI). Root samples were prepared for determining N and P by digesting the samples in concentrated sulfuric acid followed by oxidation with hydrogen peroxide (Technicon, 1977). Concentrations of N and P were measured colorimetrically using an auto-analyser (Technicon, 1975).

Classification of Root Diameter

Cleaned root samples taken over the 70 cm depth interval were classified by eye into three root diameter classes (fine, medium, coarse) based on crop type. Diameter classes for barley were defined as fine (<0.15 mm), medium (0.15-0.25 mm) and coarse (>0.25 mm). For fababean, classes were defined as fine (<0.25 mm), medium (0.25-0.35 mm) and coarse (>0.35 mm) . Sub-samples of roots from each diameter class were measured for length (l) and diameter (d) using a Zeiss Mop-Videoplan interactive image analysis system interconnected with a Hitachi video camera adapted to a Zeiss photomicroscope (Pawluk, 1987). The image analysis system included a MOP digitizer tablet, color monitor and software. This system allowed for data acquisition and computation of geometric characteristics by hand tracing structures of images with

a pen on the digitizer tablet. Traced length and diameter of individual roots were used to calculate a weighted quadratic mean diameter (d). The equation is of the form: $d = \sqrt{1/L \sum(d_i^2 l_i)}$ where d_i and l_i (i =1 to n) are respective diameters and lengths of individual roots and L is the total length of all roots (Habib, 1988).

Statistical Analyses

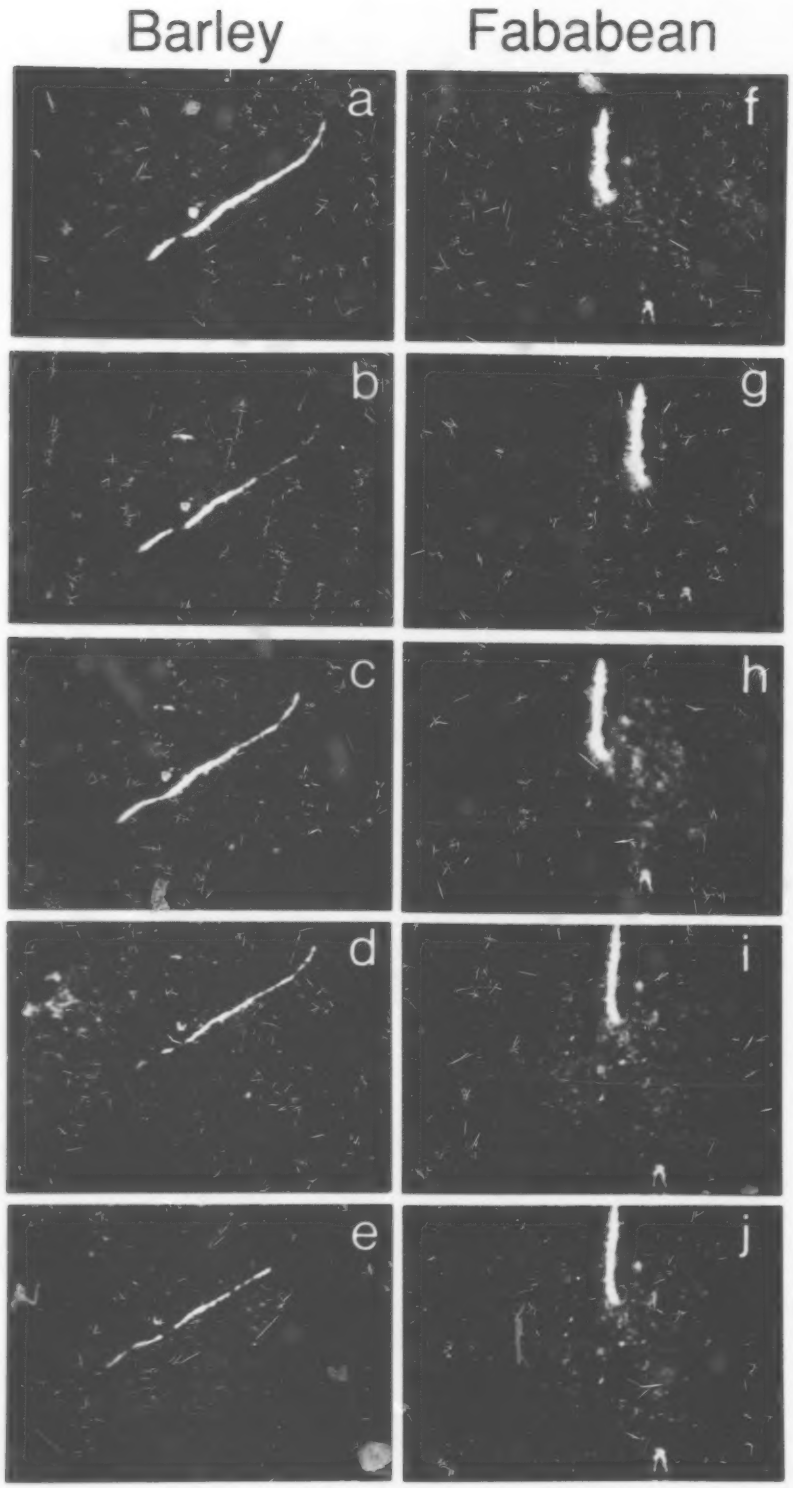
All statistical analyses were performed using PC SAS (Sas Institute, Inc. 1985). A t-test was used to compare mean differences in specific root length and diameter classes between barley and fababean ($p \leq 0.05$). The GLM procedure was used for determining ANOVA in root length intensity. The Least Significant Difference ($p \leq 0.05$) were performed for means separation of significant main effects and interactions. A 10 % significance level was selected due to the high coefficient of variation inherent in the minirhizotron technique (Upchurch, 1987; Chapter 2). Data was confirmed for normality using the W statistic ($p \leq 0.05$) of Shapiro and Wilk (1965). A Box-Cox transformation was employed as appropriate to establish homogeneity of variance (Box and Cox, 1964).

Results

Dynamics of individual barley and fababean roots

Original unregistered digitized images illustrating individual barley (a to e) and fababean (f to h) roots in various stages of growth and decomposition are presented in Plate IV.1. All observations are taken at a depth of 38 cm in barley and 31 cm for fababean within a viewing area of 2.4 cm^2 (2 cm horizontal x 1.2 cm vertical). The barley root (**1a**) had a finer diameter than the fababean root (**1f**). From the original unregistered digitized images, some decomposition of the root tip was observed at the stem growth stage (**1b**), however, there was significantly higher decomposition at the

Plate IV.1. Original unregistered digitized images in barley (**a to e**) and fababean (**f to j**) at a depth of 38 cm in barley and 31 cm for fababean within a viewing area of 2.4 cm^2 minirhizotron viewing area. Scenes for barley correspond to (**a**) tillering, (**b**) stem extension, (**c**) heading, (**d**) dough, and (**e**) ripening and in fababean correspond to (**f**) bud, (**g**) flower, (**h**) pod, (**i**) early ripening, and (**j**) late ripening growth stages. The leaf growth stage in fababean has been omitted because of absence of roots. Gridlines appear darkly toned in the images.


Barley
Fababean
a
b
c
d
e
f
g
h
i
j

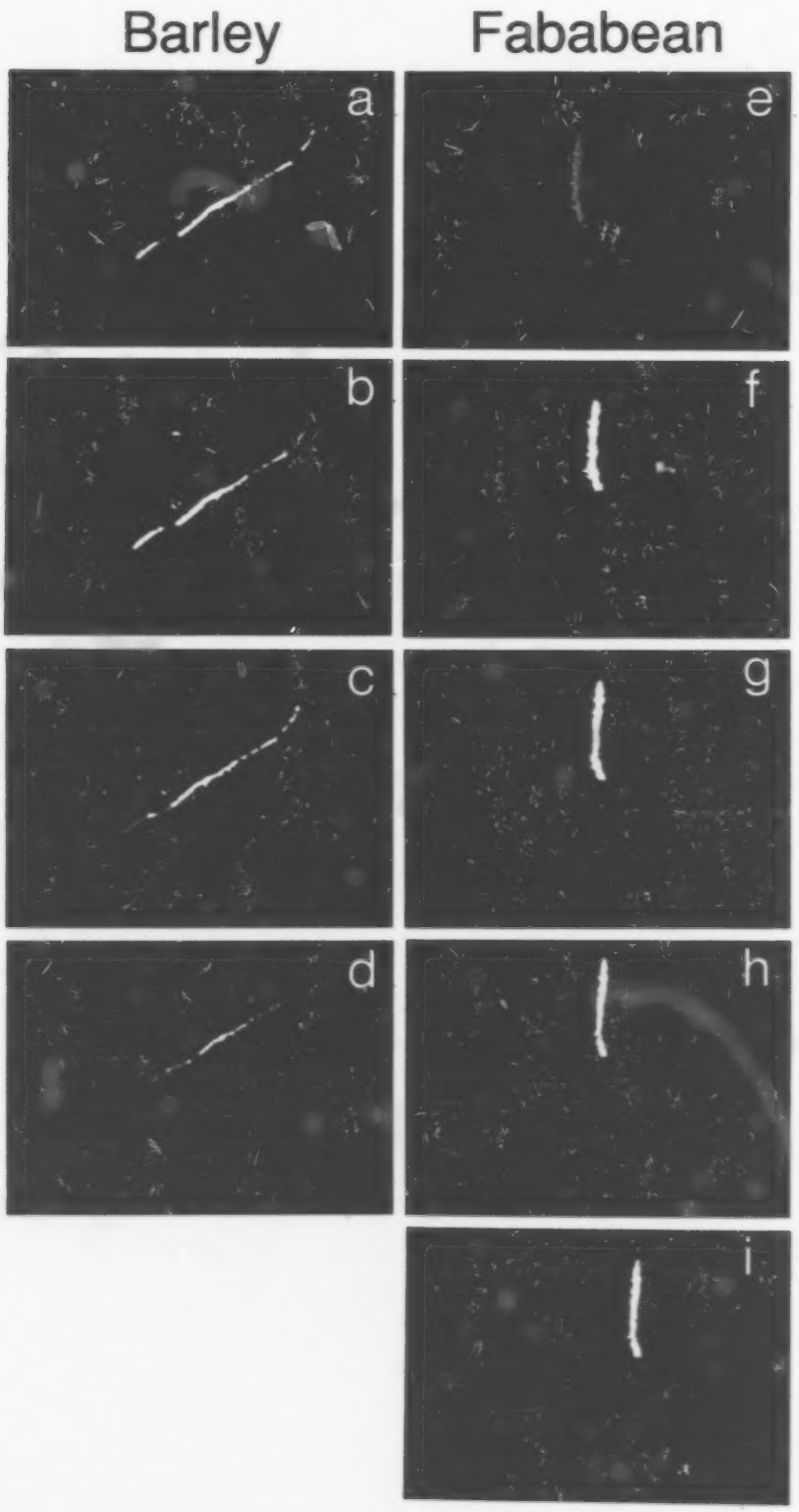
dough (**1c**) and ripening (**1d**) growth stages. This resulted in a reduction of root diameter. The barley root shifted within the viewing area at the end of the growing season (**1d**). In fababean, a root tip appeared at the bud formation stage with root hairs some distance behind the growing tip (**1f**). The root tip was extended at flowering (**1g**). Most of the root hairs disappeared at podding (**1h**) and some thinning in the roots occurred at early (**1i**) and late ripening (**1j**) growth stages.

Changes in root dynamics can be detected from a red-green color composite (Plate IV.2). An interpretative key to Plate **2** is given in Table IV.2. Distortion from a separation of red/green primary colors is more prominent in the barley (**2a** to **2d**) than fababean (**2e** to **2i**) color composites. This was most evident in registering the ripening to dough growth stage (**2d**). Barley root decomposition is detected by the appearance of a red color at stem (**2b**), dough (**2c**) and ripening (**2d**) growth stages. An increase in root growth at heading is observed by an increase in green color (**2b**). In fababean, a growing root tip appears at the bud growth stage (**2f**) which becomes elongated at flowering (**2g**). Root hairs which disappeared at the bud growth stage are indicated in red. Some decomposition was observed at late ripening growth stage (**2i**).

Table IV.2 Interpretative key for Plate IV-2.

		Color Assignments			
Image		Barley		Fababean	
Barley	Fababean	Red	Green	Red	Green
a	e	Tillering	Stem	Leaf	Bud
b	f	Stem	Heading	Bud	Flower
c	g	Heading	Dough	Flower	Bud
d	h	Dough	Ripening	Pod	Early Ripening
	i			Early Ripening	Late Ripening

Plate IV.2. Color composites in barley (**a to d**) and fababean (**a to e**) after mixing the red and green primary colors of the image display taken at a depth of 38 cm in barley and 31 cm for fababean within a viewing area of 2.4 cm^2 . The assigned colors to different growth stages (GS) in barley and fababean is given in Table IV.2. Red = decrease from GS1 to GS2; Green = increase from GS1 to GS2; and Yellow = present in GS1 and GS2.


Barley
Fababean
a
b
c
d
e
f
g
h
i

Discrimination of the root and soil by density slicing is presented in Plate IV.3 for barley (**3a** to **3e**) and fababean (**3f** to **3k**). Roots are observed in red against a blue soil matrix. Quantitative root and soil areas within 2.4 cm^2 are presented in Table IV.3. The area occupied by barley root ranged from 0.918 % at tillering to 0.368 % at the ripening growth stage. Total area of new (L_n) and dead (L_d) roots were +1.189 % and -0.821 %, respectively. In fababean, root areas ranged from 0.799 % at flowering to 0.620 % at late ripening. The total area of new roots was +0.799 % compared to -0.179 % for dead roots. Root length turnover (L_t) in barley (0.669) was 3 times higher than fababean (0.224). Incremental changes in root areas for barley were negative at stem (-0.421), dough (-0.386) and ripening (-0.014) growth stages. In contrast, changes in fababean were lower and ranged from -0.009 to -0.085 between podding to late ripening growth stages. Positive incremental changes were observed at bud (+0.729) and flowering (+0.070) growth stages in fababean.

Dynamics of barley and fababean roots in 0-70 cm depth

The effect of growth stage on mean RLI estimates was significant in barley and fababean (Table IV.4). Root length intensity increased in barley at tillering (0.21) and reached a maximum at the heading (0.37) growth stage, followed by a sharp decline at ripening (0.23) (Fig. IV.1). The absolute differences in RLI observed in barley between stem and dough growth stages were not significantly different (p=0.68), but were significantly different from the ripening growth stage. In fababean, RLI increased continuously from the leaf growth stage and peaked at early ripening (0.25). A significant difference in RLI was observed between early and late ripening growth stages (p=0.068). Barley RLI was generally higher (0.21-0.36) than fababean (0.09-0.25) for the entire 70 cm depth (Fig. IV.1).

Plate IV.3. Classified single images in barley (**a to e**) and fababean (**f to j**) after density slicing and small region conversion taken at a depth of 38 cm in barley and 31 cm for fababean within a viewing area of 2.4 cm^2 . Roots are displayed in red against a blue soil matrix. Scenes in barley correspond to (**a**) tillering, (**b**) stem extension, (**c**) heading, (**d**) dough, and (**e**) ripening and in fababean correspond to (**f**) bud, (**g**) flower, (**h**) pod, (**i**) early ripening, and (**j**) late ripening growth stages. The leaf growth stage in fababean has been omitted because of absence of roots.

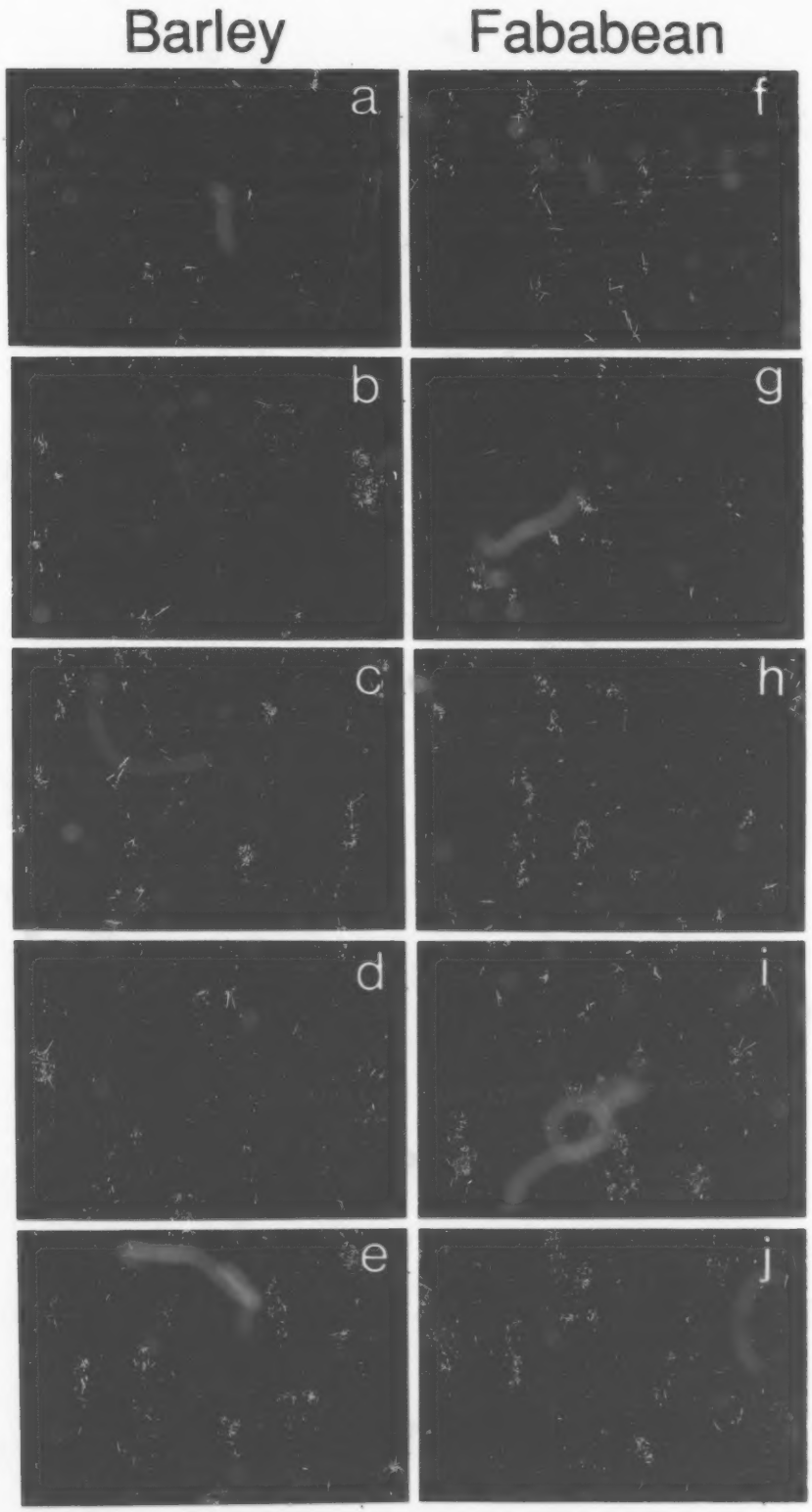

Barley
Fababean
a
f
b
g
c
h
d
i
e
j

Table IV.3 Root area (%) and turnover dynamics in barley and fababean roots as observed from classified images taken at a depth of 38 cm in barley and 31 cm for fababean within a viewing area of 2.4 cm^2 (Plate IV-3) (Red Class = Root, Blue Class = Soil Matrix)

	Red Class			Blue Class	
Growth Stage	No. of Pixels	Root Area† (%)	Area Change (%)	No. of Pixels	Soil Matrix (%)
Barley					
Tillering	2428	0.988	+ 0.988	243332	99.01
Stem	1394	0.567	- 0.421	244366	99.43
Heading	1888	0.768	+ 0.201	243872	99.23
Dough	940	0.382	- 0.386	244820	99.62
Ripening	905	0.368	- 0.014	244855	99.63
New roots (L_n)			+ 1.189		
Dead roots (L_d)			- 0.821		
Root length turnover (T_l)‡			0.690		
Fababean					
Leaf	0	0	0	245760	100.00
[illegible]	1791	0.729	+ 0.729	243969	99.27
[illegible]	1955	0.799	+ 0.070	243795	99.20
[illegible]	1754	0.714	- 0.085	244006	99.29
Early R	1546	0.629	- 0.085	244214	99.37
Late R	1523	0.620	- 0.009	244237	99.38
New roots (L_n)			+ 0.799		
Dead roots (L_d)			- 0.179		
Root length turnover (T_l)‡			0.224		

†Root Area (%) = (#of pixels in class/245760) x 100. Areas were determined after small region conversion.

‡$T_l = L_d/L_n$ where L_d (-) indicates root decomposition and L_n (+) indicates positive root growth.

Table IV.4. ANOVA† of root length intensity (cm cm^{-2}) in barley and fababean

Source of Variation	DF	Root Length Intensity	
		Barley	Fababean
Block	2	ns	ns
Depth	6	ns	ns
Error A	12		
Growth Stage	4	***	**
Error B	8		
Depth x Growth Stage	24	*	ns
Error C	48		

†The difference between means is significant at: *, p≤ 0.10; **, p≤0.01; ***, p≤0.001; ns, not significant

There was a significant growth stage and depth interaction in barley but not in fababean (Table IV.4). Root length intensity reached a maximum in barley between stem and heading growth stages and decreased at ripening for all depths (Figure IV.2). The mean difference in RLI observed at heading and ripening growth stages for the top 40 cm depth interval were different (p≤0.10). In fababean, RLI was maximum at early ripening for depths >10 cm. However, no significant differences in RLI were observed at early and late ripening growth stages.

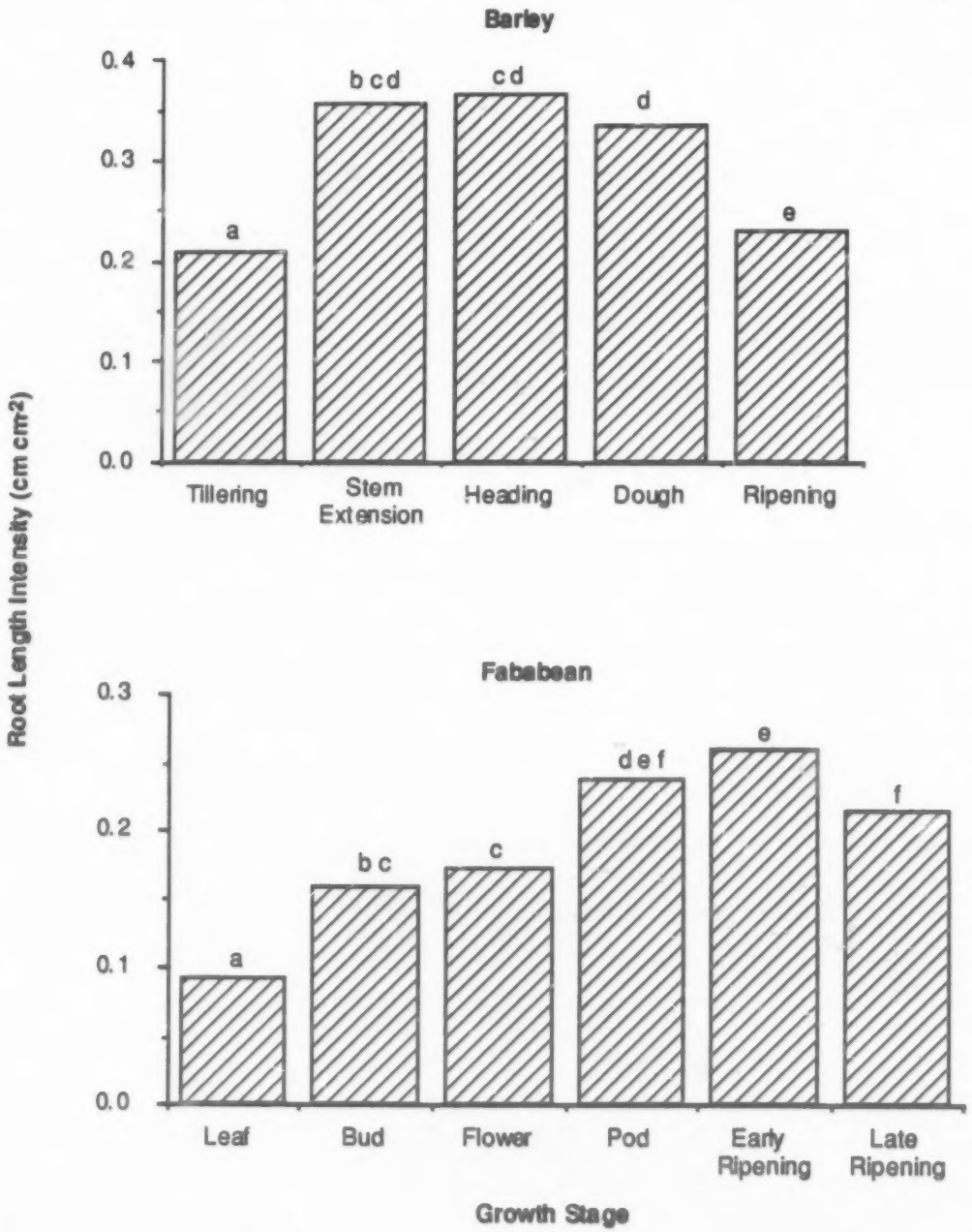


Figure IV.1. Root length intensity (RLI, cm cm^{-2}) in barley and fababean at different growth stages for the entire rooting depth (0-70 cm). The means for each growth stage bearing different letters are significantly different ($p \leq 0.05$).

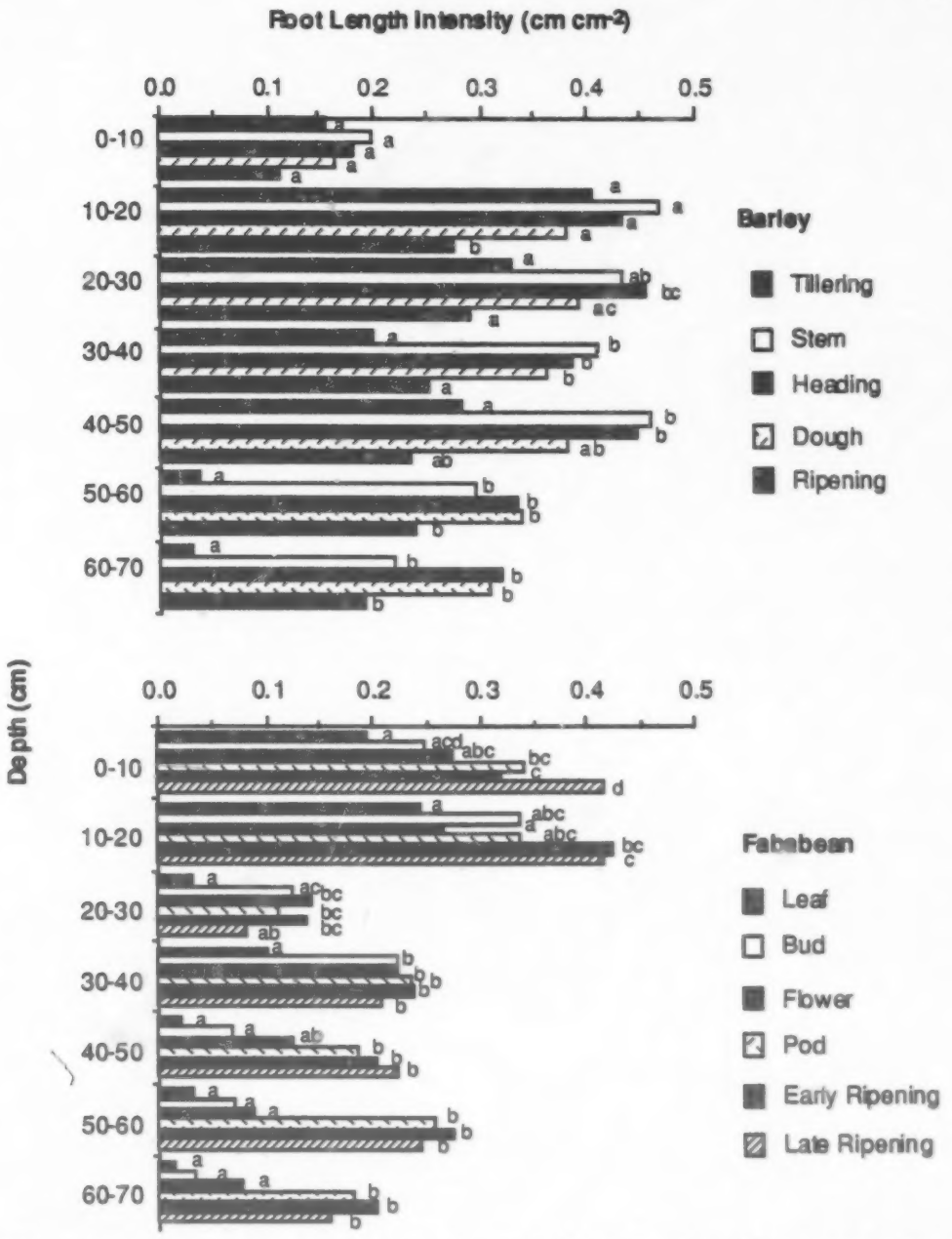


Figure IV.2. Root length intensity (RLI, cm cm^{-2}) distribution with depth at different growth stages for barley and fababean. The means for each growth stage within each depth bearing different letters are significantly different from each other ($p \leq 0.05$).

Chemical and Physical Analyses of Roots Obtained from Monoliths

Total C and P concentrations in barley roots were higher than in fababean roots. However, total N content was lower in barley than in fababean roots. This resulted in significantly higher C:N and a significantly lower C:P ratio in barley than fababean roots ($p \leq 0.05$) (Table IV.5). Root diameters for both crops were <0.5 mm and within the very fine fraction diameter class (Böhm, 1974) (Table IV.6). Barley root diameter in all the diameter classes (fine, medium, coarse) were significantly lower than those of fababean ($p \leq 0.05$). The barley root diameter measurements generally ranged between 0.13-0.28 mm compared to 0.18-0.42 mm in fababean. An overall mean diameter in barley (0.20 mm) was significantly lower than fababean (0.31 mm). Specific root length (SRL, m g^{-1}) was significantly greater for barley than fababean for all depths ($p \leq 0.05$) (Table IV.7). The overall mean specific root length for the 70 cm depth interval was significantly higher for barley than fababean (Table IV.7).

Table IV.5. Chemical analyses† of total carbon (C), nitrogen (N) and phosphorous (P) in barley and fababean roots

Crop	Total C (%)	Total N (%)	Total P (%)	C:N	C:P
Barley	40.75‡ (0.91)	1.62 (0.01)	0.11	25 (0.01)	370
Fababean	38.22 (1.30)	2.55 (0.14)	0.06	15 (0.01)	637

†Analyses determined at plant maturity

‡Means with SE in brackets among 3 measurements

Table IV.6. Mean diameter† classes for barley and fababean roots

	Diameter (mm)	
Class	Barley	Fababean
Fine	0.13 (0.01)a‡	0.18 (0.01)b
Medium	0.18 (0.01)a	0.32 (0.03)b
Coarse	0.28 (0.02)a	0.42 (0.04)b
Average	0.20 (0.01)a	0.31 (0.03)b

†Weighted quadratic diameter with SE in brackets (n=10) determined at ripening growth stages using the monolith method.

‡Values for a given crop followed by different letters in a diameter class indicate significant differences ($p \leq 0.05$) using a t-test.

Table IV.7. Specific root length (SRL, m g^{-1}) for barley and fababean measured by the monolith method†

	Specific Root Length	
Depth (cm)	Barley	Fababean
	(m g^{-1})	
0-10	146.8 (10.8)a‡	79.7 (5.2)b
10-20	163.6 (8.9)a	100.7 (3.5)b
20-30	156.2 (9.7)a	58.9 (8.5)b
30-40	179.8 (6.9)a	74.3 (11.8)b
40-50	173.0 (1.5)a	90.7 (0.5)b
50-60	166.9 (4.2)a	81.6 (10.2)b
60-70	144.1 (2.0)a	85.5 (2.4)b
Average	166.3 (6.3)a	81.7 (6.0)b

†Destructively sampled at the ripening growth stages.

‡Means with CV in brackets (n=3). Means in same row with different subscripts differ significantly ($p \leq 0.05$) using a t-test.

Discussion

The distribution of root length intensity with depth was more uniform in barley than fababean. Higher values of RLI were observed in barley than fababean in the subsurface soil layers. This is consistent with previous findings that rooting intensity is higher in cereals than legumes (Hamblin and Tennant, 1987; Gregory and Brown, 1989, Chapter 2). Differences in depth distribution are partly due to morphological differences between grass and legume root systems. The barley plant has a scattered or fibrous root system with most of is nodal roots running obliquely in the profile (Weaver, 1926; Yamauchi et al., 1987). In contrast, fababean has a superficial or tap root system with most of its root length concentrated in the top 30 cm of the soil surface (El-Shazly and El-Rassas, 1989).

Phenotypic differences in RLI were observed over the growing season. Barley has a determinate growth habit with distinctive vegetative and reproductive periods. The rapid decline in barley RLI at heading indicates that the reproductive component became a strong sink for carbohydrates. This results in a decrease in the amount of photosynthates for additional root growth (Gardner et al., 1985). In fababean, a decrease in root length intensity was not observed until early ripening. The rapid root growth up to early ripening corresponds to the vegetative growth stage of fababean. Fababean is an indeterminate crop which can contain chlorophyll in the leaves at maturity whereas the barley plant like other cereals lose their photosynthetic ability by physiological maturity (Milthorpe and Ivins, 1965). This suggests that during the vegetative growth stage in fababean, a significant partitioning of assimilate occurs above- and below-ground to encourage development of the entire plant system. Thus, it appears that the fababean has the capability to sustain or produce roots late in the growing season. There is limited information describing the allocation of carbon above- and below- ground for fababean at different growth stages in the literature.

Underestimation of rooting intensity for the top 10 cm layer has been reported and seems to be an artifact in the minirhizotron technique (Cheng et al., 1990; Vos and Groenwold, 1987; Upchurch and Ritchie, 1983; Bragg et al., 1983; Gregory, 1979; Sanders and Brown, 1979, Chapter 2). It is speculated that this may be partly due to inherent soil properties (Smucker et al., 1987), loss of resolution of roots growing horizontally near the surface (Beyrouty et al., 1987), light leaks through gaps at the minirhizotron wall (Levan et al., 1987) and temperature differences at the glass soil interface (McMichael and Taylor, 1987). Vos and Groenwold (1987) also suggested that the physical presence of the tube may cause disturbance to the water regime above the tube thus reducing root length observations in dry periods compared to the bulk soil. The magnitude of RLI underestimation in the top 10 cm layer was higher in barley than fababean. A similar observation was reported in Chapter 2. This may be due to a greater light tolerance by roots of fababean than barley. Certain species of plant roots (corn, tomato, cotton and soybean) have been observed to show greater tolerance than others (peanut) (Pearson, 1974). Even the standard procedures for eliminating light entry in minirhizotons may still result in a 50% reduction in root length in surface layers. The problem of light entry below-ground may be partially solved by using elaborate light-shielded minirhizotrons (Levan et al., 1987).

The specific root length varies between species and indicates the fineness of root systems (Fitter, 1985). Although a high specific root length does not always indicate a small root diameter, roots of low specific root length are more likely to have large diameters (Atkinson, 1989). de Willigen and van Noordwijk (1987) summarized specific root lengths for various crops under different conditions and reported values for specific root length in the range of 100-300 m g^{-1} with an average diameter of 0.2-0.3 mm. Atkinson (1989) reported average values of 300 m g^{-1} (range 5-529) for monocots and 100 m g^{-1} (range 69-750) for dicots. The values of SRL for barley (166) and fababean (82) from this study are within the range reported in the literature.

The higher values in specific root length reported in this study showed that barley has a finer root system than fababean because the mean diameter was 0.20 mm for barley roots and 0.31 mm for fababean roots.

Root length dynamics for individual barley and fababean roots within the 2.4 cm^2 minirhizotron viewing area showed similar trends as observed in the entire 70 cm depth interval. The extent of root decomposition in individual barley roots (-0.821) was greater than fababean (-0.179). Waksman and Teney (1927) suggested that 1.7% of nitrogen in decomposing rye was sufficient for microbial needs and that any nitrogen in excess of this value was rapidly mineralized. Ziemiecka and Kobus (1960) found that leguminous plant residues containing 2% or more of nitrogen are vigorously mineralized by microorganisms. The values of percentage nitrogen reported in this study for barley (1.62%) and fababean (2.55%) roots suggest that the rate of decomposition of fababean roots would be higher than barley. However, the higher turnover rate observed for the barley root indicates that the finess of the root may be an important addition to chemical characteristics such as C:N, lignin, carbohydrate (Herman et al., 1977; Dormaar et al. 1981; Muller et al., 1988) controlling the rate of decomposition. Xu and Juma (1992) observed that root length decreased more than root mass at the dough and ripening growth stages in four barley cultivars. They attributed this to the decomposition of the fine roots at the end of the growing season. Similar trends were observed with the color composite technique applied to sequential minirhizotron images. The dynamics of these fine roots are important to cycling of carbon within the soil ecosystem.

Implications

The root system of a plant is in dynamic balance with its environment through growth and decay. A higher root length intensity and specific root length for barley reported in this study arises from the length of the fine branch roots. These fine roots observed from the color composites turnover at a faster rate than the coarser fababean

roots. This indicates that the fine roots of barley rapidly decompose from the main axis, however the main axis survives to carry on its transport function (de Willigen and van Noordwijk, 1987). Finally, the higher root turnover rate indicates a higher root production in barley than fababean. This is important for understanding the impact of roots of different crops on carbon cyling in soil.

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Chapter 5. General Discussion and Conclusions

The overall objective of this study was to adapt the minirhizotron technique to study the distribution and dynamics of barley and fababean roots *in situ*. To accomplish this, an experiment was conducted in the greenhouse with barley and fababean plants grown in plywood boxes (80 cm x 80 cm x 75 cm) containing soil from the surface horizon of a Black Chernozem. The plants were seeded in a hexagonal arrangement to minimize the effects of rooting anisotropy. Minirhizotrons were installed at a 45° angle in the center of the boxes and observations were conducted to a depth of 70 cm at specified growth stages. Plants were also destructively sampled by core and monolith methods at the ripening growth stages.

Barley and fababean plants were selected in this study because their root systems are morphlogically different. Barley has a fibrous or scattered root system compared to a fleshy tap root system of fababean. The root length distribution in the two crops was compared using the minirhizotron, core and monolith techniques in Chapter 2. A color composite technique using digital image analysis for qualitatively detecting changes in root growth and decomposition from minirhizotron images was developed as described in Chapter 3. The distribution and dynamics of barley and fababean roots *in situ*. are presented in Chapter 4. A general discussion and future research are presented in this chapter.

Distribution of barley and fababean root systems

The objective of chapter 2 was to compare the results from the minirhizotron, core and monolith methods in quantifying root length distribution for barley and fababean. The overall estimates of root length density (RLD, cm cm^{-3}) by the three methods in both crops were not significantly different. However, there were significant differences in root length distributions based on a method x depth

interaction. In barley, root length estimates by the monolith method were lower in the upper 40 cm soil depth compared to the core method but were higher at all depths in fababean. The coefficient of variation of root length in fababean was observed to be lower with the monolith than core method. However, an opposite trend was found for root length measurements in barley. These observations suggested that the monolith method provided a more consistent estimate of root length in fababean than barley. Lower estimates in root length by the monolith method in the upper 40 cm layer may be due to losses in fine roots from washing a larger sample size. Additionally, incomplete separation of live roots from organic material, loss in detection of fine roots from overcrowding and the resolution limit of the video-image analyzer may have introduce erroneous estimates during automated root length measurements. These errors were probably smaller in fababean than barley because it has a coarser and less dense root system.

Regression analyses were used for comparison of the minirhizotron with the destructive methods. The *r* values between the minirhizotron and destructive methods were lower in barley (0.51-0.77) than fababean (0.46-0.52). Root length density estimated by the minirhizotron and core methods were significantly correlated ($p<0.05$), but the RLD estimates for fababean were not. It was concluded in this study that the uniform root length distribution in barley with depth may have contributed to the significant linear relationship. In contrast, the low rooting intensity for fababean observed in the subsurface soil layers may have reduced the likelihood of roots intersecting the sides of the tube. Additionally, the overestimation in RLD for fababean at lower soil depths (30 cm) may have affected the strength of the calibration relationship.

It was concluded in Chapter 2 that preferential root growth along the tube from a dominant vertical root morphology may have resulted in an overestimation of RLD in fababean compared to barley. In this study, the tubes were oriented at a 45° angle.

Yamauchi et al. (1987) found that more than 50% of the nodal roots had an angle greater than 30°. Bragg et al. (1983) found for cereals that root growth down the side of a tube occurred more in vertical tubes, but not in tubes angled at 45°. If the fababean root system morphology is more vertical than horizontal, then an angle which is slightly more horizontal (30°) may be a better placement for estimating RLD in the bulk soil. There is a need to study the root morphology of fababean by determining: (1) the number of branches, (2 the distance between branches, and (3) the angles of branching. Such studies can assist in establishing suitable orientation angles for minirhizotron tube placement.

Preferential root growth may have also been augmented by air gaps (voids) at the soil-tube interface. Several authors have reported that poor soil/tube contact may be a major problem in minirhizotron measurements, as it not only results in aberrant root growth along the tube, but also in a reduced visibility of the roots growing in the gap between the minirhizotron wall and the soil. Upchurch and Ritchie (1983) showed that the existence of these gaps can lead to the formation of bunches of roots or to tracking of roots along the surface of the tube. In soils with dense layers impeding root penetration, a tunneling effect can also be created by these gaps resulting in deeper rooting along the tube than in the undisturbed soil (Vos and Groenwold, 1987). Taylor and Böhm (1976) also found that roots proliferated more strongly when they grew along the acrylic plastic rhizotron wall than within the bulk soil, which they attributed to a poor contact between wall and soil. In this study, the soil was artificially compacted around the minirhizotron tube which may have induced voids and rapid root elongation arising from low penetration resistance. To overcome these problems, Gijsman et al. (1991) describe an inflatable minirhizotron system which allows for root observations with improved soil/tube contact. This system involves the usage of an inflatable flexible rubber wall, made from a modified motorcycle tube. By pressurizing the tube, a proper soil/tube contact is ensured so that the environmental circumstances for root

growth along the tube more closely correspond to those in the undisturbed soil. Further research is required to test whether this system will overcome the problem of root length overestimation in fababean.

In both barley and fababean crops, estimates of root length density estimated by the minirhizotron technique in the top 10 cm layer were lower than that compared to the destructive methods. Core sampling may be required in surface soil layers to obtain reliable estimates in root length density. Underestimation of RLD in the top 10 cm layer was more pronounced in barley than fababean (Chapters 2 and 4). The exact reasons for underestimation are still not clear. It has been suggested that this may be due to inherent soil properties, loss of resolution of roots growing horizontally near the surface and light leak effects through gaps at the minirhizotron wall. It is possible that the lower root tolerance for light in barley roots may have resulted in less horizontal growth and more downwards root growth in the top 10 cm layer. Further research is needed to test this hypothesis. Temperature differences at the glass soil interface might have also exacerbated heat transfer down the tube resulting in the drying and pulling away of the soil from the tube. This may have been amplified by the shrinking and swelling behaviour of the silty clay loam soil. It is a major drawback of the minirhizotron technique that estimates of RLD are lower in the uppermost soil layer where root processes are very dynamic. A scope for future research is to elucidate the exact reasons for RLD underestimation in this layer.

The heterogeneity associated with the root system and soil properties was minimized in this study by using a hexagonal planting arrangement with soil compacted artificially to a certain bulk density. Thus, the results obtained for RLD estimates in barley and fababean cannot be extrapolated to field conditions since the growing conditions are not realistic. Additionally, the pattern of root distribution from a hexagonal planting arrangement will be expected to be different as compared to seeding in rows where root density is usually highest in the center of the rows and declines

exponentially to the midrow. Nevertheless, the results obtained in Chapter 2 are an approximation of the precision and consistency in the three methods for the two genetically different crops. The methods were compared at the ripening growth stages when the entire root systems in the plants were fully developed. The conclusions reported in this study for comparing the minirhizotron technique with destructive methods may not be reliable for earlier growth stages because of a smaller and less dense root system. Furthermore, at a low rooting intensity, the probability of a root intersecting the minirhizotron is small which can often result in large CVs (>100%). Upchurch (1987) stated that "the minirhizotron should not be expected to provide a high correlation with other techniques when the root length density is low". Future research is needed to develop methods to quantify root distribution at early growth stages.

Root length intensity for barley was higher than fababean (Chapters 2 and 4). The pattern of root development is genetically controlled and can be greatly modified by physical and chemical factors in the soil environment and by plant breeding (Atkinson, 1990). There is no consistent opinion in the literature on the most favorable root size for maximum crop yield. This discrepancy is due to the fact that the size of the root system is closely related to environmental conditions (Glinski and Lipiec, 1990). An extensive root system causes plants to make more efficient use of fertilizers and water and to be less susceptible to chemical and physical stresses during the vegetative season. Such as system is more important in soils of limited water storage capacity and nutritional status. However, a plant with an extensive root system distributes more assimilates into root dry matter production than into the shoot system which can result in decreased yield. The smaller root system can be sufficient in soils of higher fertility or when such practices as irrigation and foliar fertilization are applied (de Willigen and van Noordwijk, 1987).

Dynamics of barley and fababean root systems

One of the advantages of the minirhizotron technique is that roots intersecting the minirhizotron tube can be video-recorded at the same depth over the season of growth. Thus, root growth and root decay can be closely monitored for the entire growing period with little disturbance to the natural environment. This technique represents an improvement over traditional root coring to study root dynamics because of reduced sample variance, increased frequency of measurement, ease of computerized image analysis, and perhaps most important, minimal disruption of the spatial and temporal relationships among roots and the soil. In Chapter 3, a color composite technique was developed by coupling minirhizotron observations with digital image analysis procedures. Subtle changes in root growth and decomposition were detected by assigning a separate primary color (red, green, blue) to selected growth stages and then overlayed by digital image analysis to create red-green and red-green-blue color composites. This technique was found to be effective in providing a qualitative description of barley root dynamics *in situ* within 2.4 cm^2 providing that images were registered accurately to each other. Registration was sometimes a problem because of a lack of control points and a shift in control points due to soil and root movement. This phenomena was mostly evident towards the end of the growing season. Manually selecting control points was also a subjective and interative process which can be time consuming and frustrating since reference points can have similar brightness values but may represent completely different objects (quartz grains, other soil particles, root tips and soil fauna). In addition, distinctive features or color characteristics of these objects were not easily discernible between scenes which made separation difficult. It was concluded that a compounding of these errors may have contributed to a high residual error.

The use of finely etched lines on the sides of the tube with an elaborate system of reference marks or pointers may be a means of overcoming registration fatigue

whilst increasing accuracy. In this way, the images are registered with respect to fixed control points rather than a dynamic soil matrix. This suggestion should be verified by future research. Further research is also required to determine whether movement of control points is a problem under field conditions. The higher bulk densities in the field may reduce the possibilities of soil movement and subsequent relocation of control points.

Qualitative changes in growth and decomposition of barley roots observed from the color composites confirmed quantitative estimates obtained from density slicing. The success of this technique is dependent on the proper installation of the minirhizotrons, specific soil type, operation of the micro-color camera and the quality of the video-images (e.g. head cleaning and other maintenance operations). Although superior quality images were obtained in this study, it has been reported that the transfer of the individual signals from the video camera to the video tape recorder can result in a 5-10% loss of signal resolution to stray radiation and electromagnetic noise. This may be minimized by using high quality, shielded cables and connectors (Stutte, 1990).

The quantification of root dynamics by the minirhizotron technique is based on the critical assumption that root growth and decay at the soil-tube interface is representative of bulk soil conditions. Smucker et al. (1987) commented that "no image analysis procedures are capable of producing reliable root quantification information if there is a fundamental problem with the soil-tube interface". In Chapter 2, a lower interception of roots was observed on the minirhizotron tube compared to the other methods in the top 10 cm layer where roots are usually concentrated. The factors responsible for this is purely speculative. An avenue for future research would be to compare root dynamics observed with the minirhizotron technique with destructive core sampling.

In Chapter 4, the minirhizotron technique was used to provide quantitative and qualitative information on barley and fababean root dynamics over the season of growth. Digital image analysis was used to generate red-green color composites and to estimate fine root turnover rates. Root length intensity (cm cm^{-2}) was observed to be higher in barley (0.21-0.36) than fababean (0.09-0.25) for the entire 70 cm depth interval. The RLI in barley increased at tillering (0.21) and reached a maximum at heading (0.37) followed by a sharp decline at ripening (0.23). This suggested that photosynthates are allocated to the reproductive component at heading rather than used for additional root growth. In contrast, RLI of fababean increased at the leaf growth stage (0.08) and peaked at early ripening (0.25) before decreasing. This observation indicated that fababean has the capability to sustain or produce roots late in the season. A growth stage and depth interaction in barley showed that there were significant differences in RLI ($p=0.10$). However, this was not evident in fababean ($p=0.27$).

The pattern of root dynamics for the entire 70 cm depth interval confirmed root dynamics observed from the color composites for individual barley and fababean roots. The extent of root decomposition in barley (-0.821) was found to be greater than fababean (-0.179) over the growing season. Turnover of fine barley roots (0.669) was estimated to be 3 times greater than fababean (0.224). It was concluded that a higher root turnover rate in barley than fababean is probably due to its fine root diameter rather than individual chemical characteristics. Although root diameter and specific root length measurements indicated that barley roots were finer than fababean, future work is needed to establish the longevity of individual roots of varying diameter. This will assist in predicting the effects of size changes of individual roots on the productivity of the root system. More observations will help to clarify the gaps in our knowledge regarding the gradual process of growth, death and decomposition in barley and fababean roots. Harper et al. (1991) stated that in order for the demography of roots to be a complete descriptor, it is necessary to analyse: (1) the rate at which whole roots

are born and die; and (2) the rate at which different parts of a single root make the transition from one phase and type of activity to another (i.e., a single root has its own demography and its own age structure). These possibilities may be explored with the minirhizotron and color composite image analysis techniques.

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Chapter 6. Appendices

Appendix 6.1. Some chemical properties of the Orthic Black Chernozem soil (Typic Cryoboroll) in the plow layer (0-10 cm depth)

Depth (cm)	pH (1:2 soil:water)	NO3-N ($\mu g\ g^{-1}$)	NH4-N ($\mu g\ g^{-1}$)	PO4-P ($\mu g\ g^{-1}$)
0-10	6.4	21.00	1.37	12.99
	6.5	19.98	1.66	13.14
	6.3	20.20	2.43	13.39

Appendix 6.2. Description of specified growth stages in barley and fababean using Zadoks and EPPO code, respectively.

Days after Seeding	Growth Code	Growth stage Description
Barley		
21	15	Tillering (1-2 tillers present)
35	39	Stem Elongation (flag leaf ligule just visible)
39	56	Heading (3/4 of inflorescence emerged)
54	87	Dough (hard)
68	92	Ripening (caryopsis extremely hard)
Fababean		
21	35	Leaf (8 leaves unfolded)
35	53	Bud formation (first flower racemes visible at shoot tip)
39	63	Flowering (>3 flower racemes/plant in bloom)
54	74	Pods visible in the middle inflorescence
68	78	Early ripening of pods (green ripeness)
82	87	Late ripening (2/3 of all pods darkly colored)

Appendix 6.3. Minirhizotron root intersects in barley at specified growth stages

Depth Interval (cm)	Growth Stage				
	Tillering	Stem Extension	Heading	Dough	Ripening
0-10	3.0	2.5	3.0	3.0	2.5
	6.5	12.5	9.0	6.5	4.5
	7.5	6.5	8.0	8.5	5.0
10-20	19.0	17.5	14.0	14.5	11.0
	14.5	20.0	19.0	16.5	11.5
	10.0	13.0	13.5	10.0	7.0
20-30	8.0	11.5	12.5	16.5	6.5
	9.0	16.0	15.5	8.5	10.5
	18.5	19.0	21.0	17.0	14.5
30-40	3.0	11.5	11.5	7.5	4.0
	18.0	21.0	16.0	17.5	13.0
	0.5	13.0	14.0	14.0	10.5
40-50	10.0	21.5	22.5	19.5	10.5
	15.0	21.5	15.5	14.0	9.5
	5.5	7.0	21.0	7.5	5.5
50-60	1.5	14.0	13.5	15.5	8.5
	1.5	13.0	12.5	12.0	12.0
	1.5	9.5	9.5	9.0	5.0
60-70	2.5	11.0	7.0	7.0	4.0
	0.0	11.5	17.0	17.5	11.5
	1.0	4.5	10.0	8.5	5.0

Appendix 6.4. Minirhizotron root intersects in fababean at specified growth stages

Depth Interval (cm)	Growth Stage					
	Leaf and Stem	Bud	Flower	Pod	Early Ripening	Late Ripening
0-10	8.5	10.5	11.5	13.5	12.5	5.5
	1.0	1.5	2.0	1.0	3.5	0.0
	11.5	15.0	6.0	22.0	18.0	12.0
10-20	8.5	11.0	5.0	10.5	7.5	3.5
	4.0	10.0	8.5	8.5	20.5	27.0
	14.0	15.5	15.0	17.5	17.0	14.0
20-30	0.5	3.0	3.5	3.0	4.0	1.5
	1.0	4.5	4.5	3.0	4.0	4.0
	2.0	6.0	7.0	6.5	7.0	3.5
30-40	2.5	4.0	3.5	4.5	5.0	3.5
	4.0	14.0	14.0	13.5	13.0	10.0
	4.5	6.0	6.5	7.0	8.0	8.5
40-50	1.0	1.5	2.0	3.0	4.0	3.5
	0.5	1.5	2.5	2.0	4.0	5.5
	1.0	4.5	9.0	15.0	14.0	15.0
50-60	2.0	3.0	4.0	5.5	4.0	4.0
	1.5	1.0	1.0	5.5	10.5	8.5
	0.0	4.0	4.5	17.0	15.5	14.0
60-70	0.0	0.0	0.5	0.5	1.0	1.5
	1.5	3.5	6.0	9.0	12.5	10.5
	0.5	0.5	2.0	10.5	8.5	5.5

Appendix 6.5. Total length of root (cm) obtained by minirhizotron, core and monolith methods at ripening growth stages in barley and fababean†

Method	Depth Interval (cm)						
	0-10	10-20	20-30	30-40	40-50	50-60	60-70
Barley							
Minirhizotron	1.9635	8.6394	5.1051	3.1416	8.2467	6.6759	3.1416
	3.5343	9.0321	8.2467	10.2102	7.4613	9.4248	9.0321
	3.9270	5.4978	11.3883	8.2467	4.3197	3.9270	3.9270
Core	1468.289	715.346	981.485	842.240	1171.073	846.700	552.630
	1015.435	664.170	1001.598	764.276	933.052	353.626	518.117
	1230.866	504.228	795.441	593.555	245.425	186.363	148.103
Monolith	5659.733	799.887	3253.902	618.545	1875.987	435.670	340.457
	5021.988	1377.673	2925.936	1047.888	3273.706	2184.130	1421.488
	5256.307	2375.930	3057.210	556.010	3867.591	2180.436	261.649
Fababean							
Minirhizotron	4.3197	2.7489	1.1781	2.7489	2.7489	3.1416	1.1781
	0.0000	21.2058	3.1416	7.8540	4.3197	6.6759	8.2467
	9.4248	10.9956	2.7489	6.6759	11.7810	10.9956	4.3197
Core	813.026	232.904	141.007	173.096	145.327	90.405	39.207
	871.076	243.136	148.720	122.400	144.093	103.673	28.149
	657.210	489.668	354.215	399.600	120.643	169.702	20.106
Monolith	5416.920	1949.140	1784.953	1580.079	1287.575	392.780	257.947
	5929.701	2585.983	1333.549	1508.806	1213.524	295.281	148.058
	6395.875	1591.478	1030.554	819.814	950.639	394.944	113.546

†Minirhizotron soil volume = 0.48 cm^3
Core volume = 503 cm^3
Monolith volume = 3000 cm^3

Appendix 6.6. Root length density (RLD, cm cm^{-3}) obtained by minirhizotron, core and monolith methods at ripening growth stages in barley and fababean

Method	Depth Interval (cm)						
	0-10	10-20	20-30	30-40	40-50	50-60	60-70
Barley							
Minirhizotron	0.351	1.543	0.912	0.561	1.473	1.192	0.561
	0.631	1.613	1.473	1.823	1.332	1.683	1.613
	0.701	0.982	2.034	1.473	0.771	0.701	0.701
Core	2.921	1.423	1.953	1.676	2.330	1.684	1.099
	2.020	1.321	1.993	1.520	1.856	0.704	1.031
	2.449	1.003	1.582	1.181	0.488	0.371	0.295
Monolith	1.887	0.267	1.085	0.206	0.625	0.145	0.113
	1.674	0.459	0.975	0.349	1.091	0.728	0.474
	1.752	0.792	1.019	0.185	1.289	0.727	0.087
Fababean							
Minirhizotron	0.771	0.491	0.210	0.491	0.491	0.561	0.210
	0.000	3.787	0.561	1.403	0.771	1.192	1.473
	1.683	1.964	0.491	1.192	2.104	1.964	0.771
Core	1.617	0.463	0.281	0.344	0.289	0.180	0.078
	1.733	0.484	0.296	0.244	0.287	0.206	0.056
	1.307	0.974	0.705	0.795	0.240	0.338	0.040
Monolith	1.806	0.650	0.595	0.527	0.429	0.131	0.086
	1.977	0.862	0.445	0.503	0.405	0.098	0.049
	2.132	0.530	0.344	0.273	0.317	0.132	0.038

Appendix 6.7. Root length intensity (RLI, cm cm^{-2}) obtained by the minirhizotron technique in barley at specified growth stages

Depth Interval (cm)	Growth Stage				
	Tillering	Stem Extension	Heading	Dough	Ripening
0-10	0.08	0.07	0.08	0.08	0.07
	0.18	0.35	0.25	0.18	0.13
	0.21	0.18	0.22	0.24	0.14
10-20	0.53	0.49	0.39	0.41	0.31
	0.41	0.56	0.53	0.46	0.32
	0.28	0.36	0.38	0.28	0.20
20-30	0.22	0.32	0.39	0.46	0.18
	0.25	0.45	0.53	0.24	0.29
	0.52	0.53	0.38	0.48	0.41
30-40	0.08	0.27	0.32	0.21	0.11
	0.51	0.60	0.45	0.49	0.36
	0.01	0.36	0.39	0.39	0.29
40-50	0.28	0.70	0.63	0.55	0.29
	0.42	0.55	0.43	0.39	0.27
	0.15	0.13	0.28	0.21	0.15
50-60	0.04	0.34	0.38	0.43	0.24
	0.04	0.28	0.35	0.34	0.34
	0.04	0.27	0.27	0.25	0.14
60-70	0.07	0.18	0.20	0.20	0.11
	0.00	0.34	0.48	0.49	0.32
	0.03	0.14	0.28	0.24	0.14

Appendix 6.8. Root length intensity (RLI, cm cm^{-2}) obtained by the minirhizotron technique in fababean at specified growth stages

Depth Interval (cm)	Growth Stage					
	Leaf and Stem	Bud	Flower	Pod	Early Ripening	Late Ripening
0-10	0.24	0.29	0.32	0.38	0.35	0.15
	0.03	0.04	0.06	0.03	0.10	0.00
	0.32	0.42	0.45	0.62	0.51	0.34
10-20	0.24	0.31	0.14	0.29	0.21	0.10
	0.11	0.28	0.24	0.24	0.58	0.76
	0.39	0.43	0.42	0.49	0.48	0.39
20-30	0.01	0.08	0.10	0.08	0.11	0.04
	0.03	0.13	0.13	0.08	0.11	0.11
	0.06	0.17	0.20	0.18	0.20	0.10
30-40	0.07	0.11	0.10	0.13	0.14	0.10
	0.11	0.39	0.39	0.38	0.36	0.28
	0.13	0.17	0.18	0.20	0.22	0.24
40-50	0.03	0.04	0.06	0.08	0.11	0.10
	0.01	0.04	0.07	0.06	0.11	0.28
	0.03	0.13	0.25	0.42	0.39	0.24
50-60	0.06	0.08	0.11	0.15	0.11	0.11
	0.04	0.03	0.03	0.15	0.29	0.24
	0.00	0.11	0.13	0.48	0.43	0.39
60-70	0.00	0.00	0.01	0.01	0.03	0.04
	0.04	0.10	0.17	0.25	0.34	0.29
	0.01	0.01	0.06	0.29	0.24	0.15

Appendix 6.9. Percentage root mass distribution obtained by minirhizotron, core and monolith methods at ripening growth stages in barley and fababean

Method	Depth Interval (cm)						
	0-10	10-20	20-30	30-40	40-50	50-60	60-70
Barley							
Core							
	34.444	15.024	22.632	20.223	25.231	15.483	12.501
	26.416	6.996	23.090	20.567	21.026	6.614	12.692
	35.236	14.032	21.248	14.077	5.755	6.286	3.364
Monolith	42.876	6.505	28.377	4.113	12.270	3.166	2.692
	36.019	6.820	15.281	4.654	16.748	11.553	8.924
	32.137	8.865	18.392	3.345	22.895	12.556	1.810
Fababean							
Core	42.638	13.307	11.650	12.755	12.432	4.697	2.525
	53.398	14.360	9.938	6.467	6.372	7.798	1.639
	26.512	18.158	17.348	18.926	6.905	11.210	0.958
Monolith	48.980	12.672	13.814	11.020	8.833	2.942	1.738
	43.346	15.597	16.844	11.070	8.747	3.248	1.161
	52.022	10.670	14.980	11.983	6.877	2.540	0.922

Appendix 6.10. Percentage root length distribution obtained by minirhizotron, core and monolith methods at ripening growth stages in barley and fababean

Method	Depth Interval (cm)						
	0-10	10-20	20-30	30-40	40-50	50-60	60-70
Barley							
Minirhizotron	5.319	23.404	3.830	8.511	22.340	18.085	8.511
	6.207	15.862	14.483	17.931	13.103	16.552	15.862
	9.524	13.333	27.619	20.000	10.476	9.524	9.524
Core	25.191	12.273	16.839	14.450	20.092	14.527	9.481
	22.609	14.788	22.301	17.017	20.775	7.874	11.536
	33.231	13.613	21.475	16.025	6.626	5.301	3.998
Monolith	45.589	6.616	25.061	4.764	14.448	3.355	2.622
	29.108	7.985	16.959	6.074	18.975	12.660	8.239
	29.942	13.534	17.415	3.167	22.031	12.421	1.490
Fababean							
Minirhizotron	23.913	15.217	6.522	15.217	15.217	17.391	6.522
	0.000	41.221	6.107	15.267	8.397	12.977	16.031
	16.552	9.310	4.828	11.724	20.690	19.310	7.586
Core	49.727	14.245	8.624	10.587	8.889	5.529	2.398
	52.435	14.636	8.952	7.368	8.674	6.241	1.694
	29.723	22.145	16.020	18.072	5.456	7.675	0.909
Monolith	42.756	15.385	14.089	12.472	10.163	3.100	2.036
	45.561	19.869	10.246	11.593	9.324	2.269	1.138
	56.616	14.088	9.122	7.257	8.415	3.496	1.005

Appendix 6.11. Specific root length (m g^{-1}) obtained by the monolith method in barley and fababean at ripening growth stages.

	Depth Interval (cm)						
Crop	0-10	10-20	20-30	30-40	40-50	50-60	60-70
Barley							
	67.932	94.481	79.367	88.076	89.539	82.000	91.147
	89.762	108.792	51.950	89.432	91.037	59.653	83.649
	81.528	98.911	45.620	45.369	91.672	103.119	81.688
Fababean							
	152.512	142.076	132.488	173.749	176.647	159.004	146.119
	121.745	176.399	167.196	196.602	170.683	165.089	139.089
	166.286	172.469	169.000	169.000	171.740	176.554	146.994

Appendix 6.12. Fractionation of above ground components in barley and fababean at ripening growth stages

Component	Replicate # 1	2	3
Barley			
No. of plants	89	90	90
No. of spikes	182	173	156
Mean shoot length (cm)/plant	38.4	40.3	39.4
Mean no. tillers/plant	3.3	3.0	3.0
Kernel weight (g)	85	140.3	120.7
Dry weight (stems + leaves, g)	116.9	140.7	133.3
Shoot:Root ratio	25.8	25.3	24.5
Fababean			
No. of plants	27	25	27
No. of pods	104	71	80
No. of seeds	165	108	101
Seed weight (g)	122.8	71.7	73.4
Dry weight (stems + leaves, g)	144	205	232
Shoot:Root ratio	54.8	57.2	67.0

Appendix 6.13. Root dry weight (mg) in barley and fababean at ripening growth stage using core and monolith methods

	Depth Interval (cm)						
Method	0-10	10-20	20-30	30-40	40-50	50-60	60-70
Barley							
Core	0.0901	0.0393	0.0592	0.0529	0.0660	0.0405	0.0327
	0.0691	0.0183	0.0604	0.0538	0.0550	0.0173	0.0332
	0.0796	0.0317	0.0480	0.0318	0.0130	0.0142	0.0076
Monolith	0.3711	0.0563	0.2456	0.0356	0.1062	0.0274	0.0233
	0.4125	0.0781	0.1750	0.0533	0.1918	0.1323	0.1022
	0.3161	0.0872	0.1809	0.0329	0.2252	0.1235	0.0178
Fababean							
Core	0.0926	0.0289	0.0253	0.0277	0.0270	0.0102	0.0055
	0.1123	0.0302	0.0209	0.0136	0.0134	0.0164	0.0034
	0.0622	0.0426	0.0407	0.0444	0.0162	0.0263	0.0022
Monolith	0.7974	0.2063	0.2249	0.1794	0.1448	0.0479	0.0283
	0.6606	0.2377	0.2567	0.1687	0.1333	0.0495	0.0177
	0.7845	0.1609	0.2259	0.1807	0.1037	0.0383	0.0139

Appendix 6.14. ANOVA of root length density (cm cm^{-3}) in barley and fababean at ripening growth stages obtained by minirhizotron, core and monolith methods

Source	DF	SS	MS	F	Pr >F
Barley					
Block (B)	2	0.80489	0.40244	0.59	0.5981
Method(M)	2	5.33635	2.66817	3.89	0.1155
B*M	4	2.74651	0.68663	5.79	0.0011
Depth (D)	6	5.72703	0.95450	8.04	0.0001
M*D	12	7.15890	0.59657	5.03	0.0001
B*M*D	36	4.27142	0.11865		
Fababean					
Block (B)	2	1.55726	0.77863	1.19	0.3938
Method (M)	2	3.84291	1.92145	2.93	0.1646
B*M	4	2.62337	0.65584	3.26	0.0223
Depth (D)	6	9.04103	1.50684	7.48	0.0001
M*D	12	7.61556	0.63463	3.15	0.0038
B*M*D	36	7.24900			

Appendix 6.15. ANOVA of root mass density (mg cm^{-3}) in barley and fababean at ripening growth stages obtained by minirhizotron, core and monolith methods

Source	DF	SS	MS	F	Pr >F
Barley					
Block (B)	2	0.00293	0.00146	0.65	0.6064
Method (M)	1	0.01585	0.01585	7.02	0.1178
B*M	2	0.00452	0.00226	6.33	0.0062
Depth (D)	6	0.05200	0.00867	24.31	0.0001
M*D	6	0.00331	0.00055	1.55	0.2061
B*M*D	24	0.00856	0.00035		
Fababean					
Block (B)	2	0.00001	0.00005	0.47	0.6787
Method (M)	1	0.00136	0.00136	13.27	0.0678
B*M	2	0.00020	0.00010	0.23	0.7952
Depth (D)	6	0.16511	0.02752	62.16	0.0001
M*D	6	0.00777	0.00129	2.92	0.0276
B*M*D	24	0.01063	0.00044		

Appendix 6.16. ANOVA of root length intensity (cm cm^{-2}) in barley and fababean obtained by the minirhizotron over the growing season

Source	DF	SS	MS	F	Pr >F
Barley					
Block (B)	2	0.18120	0.09060	1.68	0.0005
Depth (D)	6	0.72102	0.12017	2.23	0.0001
B*D	12	0.64576	0.05381	8.65	0.0001
Growth Stage (S)	4	0.46707	0.11677	18.76	0.0001
B*S	8	0.03275	0.00409	0.66	0.7254
D*G	24	0.22906	0.00954	1.53	0.1030
B*D*S	48	0.29876	0.00622		
Fababean					
Block (B)	2	0.44447	0.00005	3.84	0.0515
Depth (D)	6	0.77799	0.00136	2.24	0.1107
B*D	12	0.69522	0.00010	9.93	0.0001
Growth Stage (S)	5	0.37897	0.02752	12.99	0.0001
B*S	10	0.18259	0.00129	3.13	0.0028
D*S	30	0.20921	0.00697	1.19	0.2741
B*D*S	60	0.35017	0.00584		

Appendix 6.17. Diameter (mm) and root length (cm) measurements for individual barley and fababean roots

	Diameter (mm)			Root Length (cm)		
Replicate	Fine	Medium	Large	Fine	Medium	Large
Barley						
1	0.131	0.200	0.319	25.155	45.186	63.248
2	0.129	0.126	0.285	21.023	41.263	45.734
3	0.097	0.212	0.283	30.974	24.628	42.726
4	0.121	0.200	0.282	31.247	36.837	44.337
5	0.106	0.223	0.264	27.102	46.528	41.120
6	0.097	0.201	0.301	37.030	33.734	68.107
7	0.171	0.198	0.263	32.515	57.280	56.452
8	0.091	0.158	0.270	19.205	42.728	43.179
9	0.152	0.150	0.198	17.996	51.437	53.2[illegible]
10	0.141	0.136	0.248	36.381	27.749	37.990
Fababean						
1	0.230	0.269	0.513	25.312	38.088	38.385
2	0.174	0.386	0.314	23.364	60.551	63.663
3	0.144	0.237	0.414	24.254	44.715	48.682
4	0.169	0.336	0.636	23.813	52.954	50.312
5	0.155	0.379	0.408	29.410	31.761	44.117
6	0.175	0.421	0.417	27.288	26.805	67.025
7	0.142	0.256	0.360	27.091	34.260	38.906
8	0.229	0.242	0.396	29.040	28.551	37.711
9	0.165	0.299	0.301	32.019	48.534	44.632
10	0.145	0.277	0.308	29.027	34.795	33.918